

https://dspace.org/

Cytological and cytogenetical studies on the multimammate mouse, Praomys (Mastomys) natalensis

Hallett, Janet Mary

2015-01-23
http://hdl.handle.net/10539/16713

Downloaded from a DSpace repository. https://dspace.org/

CYTOLOGICAL AND CYTOGENETICAL STUDIES ON THE MULTIMAMMATE MOUSE, PRAOMYS (MASTOMYS) NATALENSIS

Janet Mary Hallett

A Dissertation Submitted to the Faculty of Science
University of the Witwatersrand, Johannesburg
for the Degree of Master of Science

Durban, 1977

To my husband, Dr. A. F. Hallett

and children, Andrew and Stephen

ABSTRACT

A cytological and cytogenetical study on the multimammate mouse, Praomys (Mastomys) natalensis from various parts of southern Africa was carried out in order to establish the chromosome pattern in wild populations. Bone marrow suspensions from specimens pretreated with colchicine were used to obtain mitotic metaphase plates. Chromosomes were counted in the plates and karyotypes were constructed from photomicrographs of clear plates. Meiotic metaphase was studied in testicular suspensions from the male specimens.

Mastomys from the south (eastern Cape Province) and the central interior (Lesotho, northern Orange Free State, northern Cape Province and southern Transvaal) were found to have 2n = 36 chromosomes. Only the group from the northern Cape Province had a slightly different karyotype from the rest due to a pericentric inversion affecting one pair of autosomes.

Mastomys from the more northerly areas (South West Africa, Rhodesia, northern and eastern Transvaal) were found to have 2n = 32 chromosomes. The specimens from Rhodesia had a slightly different karyotype from the other 2n = 32 chromosome groups probably due to a pericentric inversion affecting one pair of autosomes. Mastomys with 2n = 32 chromosomes have previously only been known as far south as Congo-Brazzaville in central Africa.

From comparisons of the karyotypes in the two groups within southern Africa it appears that centric fusion between two non-homologous acrocentric autosomes was responsible for reduction in chromosome number by one pair.

The second reduction was possibly due to the translocation of the long arm of an acrocentric autosome onto a submetacentric autosome. The Y chromosome in the 2n = 36 chromosome group is submetacentric, while in the 2n = 32 chromosome group it is acrocentric. This change in the position of the centromere was probably due to a pericentric inversion converting the submetacentric Y into an acrocentric Y.

Examination of the XY bivalent in meiotic metaphase plates showed *part side by side association in some plates and terminal association* in others. From this observation it appears to the author that there was a translocation of a pair of homologous autosomes onto the X and Y chromosomes *in an ancestor common to both groups.*

Behavioural differences were noted between the two chromosomally polymorphic groups. The 2n = 32 chromosome group was much more aggressive than the 2n = 36 chromosome group.

Laboratory hybridization between the two groups was achieved. The hybrids obtained were sterile and had a chromosome count of 2n = 34 made up of a haploid set of chromosomes contributed by each parent. Possible causes of sterility were investigated.

Testes in male hybrids were smaller than those of normal breeding males. Histological sections of the hybrid testes showed a reductio n the size of the tubules and within the tubules there was a lack of spermatozoa, spermatids, secondary spermatocytes and post-pachytene stages of primary spermatocytes. In the female hybrid the ovaries were slightly larger than those in normal breeding Mastomys females, while the uteri were almost double the weight of those in breeding specimens. No oocytes surrounded

by follicle cells were seen in histological sections of the female hybrid ovaries.

This chromosome polymorphism considered together with the production of sterile hybrids suggests very strongly that there are two separate species of Mastomys in southern Africa. The cytogenetic findings in these Mastomys are discussed in relation to Mastomys in other parts of Africa.

ACKNOWLEDGEMENTS

This study was begun during my employment in the Department of Anatomy of the Medical School at the University of the Witwatersrand.

I wish to record my sincere thanks to both Professor P.V. Tobias and Dr. C. Wallace for their constant advice and encouragement during the course of this project and for critically reading the manuscript.

This investigation would not have been possible but for the many people who supplied the live specimens. For these my thanks are due to the Technical Field Staff of the State Department of Health for most of the mice from South Africa, to Mr. C.G. Coetzee for the mice from South West Africa, to Dr. T. Choate for those from Rhodesia and to Dr. F. Maurer for the mice from Lesotho.

I am also indebted to the staff of the Medical Ecology Centre, Johannesburg, particularly Mrs. H. Keogh, for their assistance with identification and for providing accommodation for my mice. The map of Praomys (Mastomys) natalensis distribution was provided by their Map Unit.

The National Institute for Occupational Diseases, Johannesburg assisted with the histological preparations. Photographs were printed by the Medical Illustration Department of the University of Natal, Durban.

Finally, I would like to thank my family for their support while working on this project, and my mother, Mrs. B. Fincham, for giving up her valuable time to type this dissertation.

CONTENTS

CHAPTER			PAGE
1	INTRODUCTION		1
2	MATERIALS AND METHODS		7
	2.1 Materials		7
	2.1.1 Localities investigated		7
	2.1.2 Mapping		9
	2.2 Methods		10
	2.2.1 Brief background to techniques in use for chromosome study from the bone marrow of small mammals		10
	2.2.2 Cytological techniques		11
	2.2.3 Karyotyping methods		15
	2.2.4 Investigations of meiotic activity ..		16
	2.2.5 Histological techniques		17
3	RESULTS OF CHROMOSOME STUDIES IN PRAOMYS (MASTOMYS) NATALENSIS IN SOUTHERN AFRICA ..		18
	3.1 Mitotic Studies		18
	3.1.1 Specimens from the eastern Cape Province		18
	3.1.2 Specimens from the northern Cape Province		19
	3.1.3 Specimens from the northern Orange Free State		22
	3.1.4 Specimens from the southern Transvaal		25
	3.1.5 Specimens from the eastern Transvaal ..		28
	3.1.6 Specimens from the northern Transvaal		31
	3.1.7 Specimens from South West Africa ..		34
	3.1.8 Specimens from Rhodesia		37
	3.1.9 Specimens from Lesotho		43
	3.2 Meiotic Studies		45
	3.2.1 Meiosis in the 2n = 36 chromosome group		48
	3.2.2 Meiosis in the 2n = 32 chromosome group		52
4	DISCUSSION OF CHROMOSOME FINDINGS IN PRAOMYS (MASTOMYS) NATALENSIS IN SOUTHERN AFRICA		56
	4.1 Karyotypes of the 2n = 36 Chromosome Population		57
	4.2 Karyotypes of the 2n = 32 Chromosome Population		60
	4.3 Comparison between the Karyotypes of the Two Groups		61
	4.4 External Morphology and Behaviour of the Two Chromosome Groups		65

		PAGE
5	BREEDING EXPERIMENTS AND CONSEQUENT INVESTIGATIONS	67
5.1	Materials	67
5.2	Breeding Methods	68
5.3	Results of Cross Breeding	69
5.4	Phenotypic Appearance of the Hybrid ..	69
5.5	Chromosome Investigations in the Hybrid ..	70
5.6	Investigation into the Infertility of the Hybrids	75
5.6.1	Male hybrids	78
5.6.1.1	Histological sections ..	79
5.6.2	Female hybrids	84
5.6.2.1	Histological sections ..	85
5.7	Discussion	88
5.7.1	Mechanism of breakdown in meiosis ..	90
5.7.2	Male hybrids in some other mammals ..	91
5.7.3	Female hybrids in some other mammals	92
5.7.4	Systematic implications of results ..	93
5.8	Conclusion	95
6	GENERAL DISCUSSION AND CONCLUSIONS ..	96
6.1	Karyotypes of 2n = 32 Chromosome Group in Sub-Saharan Africa	97
6.2	Karyotypes of 2n = 36 Chromosome Group in South Africa	100
6.3	Karyotypes of 2n = 38 Chromosome Group in West Africa	102
6.4	Relationship between the Different Chromosome Groups	104
6.5	Geographic Distribution	107
6.6	Behavioural and Other Differences in Mastomys with Different Chromosome Complements ..	107
6.7	Chromosome Rearrangements and Speciation ..	110
6.8	Cytotaxonomy of Mastomys in Relation to Disease	113
	ADDENDUM	115
	REFERENCES	117

LIST OF FIGURES

		PAGE
Fig. 1.	Map of Praomys (Mastomys) natalensis distribution in southern Africa.	6
Fig. 2.	Diagram illustrating the quarter-degree square system of mapping. Kuruman is indicated by the code 2723 Ad which is the shaded square.	9
Fig. 3.	Karyotype of Praomys (Mastomys) natalensis ♂ M7 from the eastern Cape Province.	20
Fig. 4.	Karyotype of Praomys (Mastomys) natalensis ♀ M8 from the eastern Cape Province.	21
Fig. 5.	Karyotype of Praomys (Mastomys) natalensis ♂ M23 from the northern Cape Province.	23
Fig. 6.	Karyotype of Praomys (Mastomys) natalensis ♀ M24 from the northern Cape Province.	24
Fig. 7.	Karyotype of Praomys (Mastomys) natalensis ♂ M62 from the northern Orange Free State.	26
Fig. 8.	Karyotype of Praomys (Mastomys) natalensis ♀ M64 from the northern Orange Free State.	27
Fig. 9.	Karyotype of Praomys (Mastomys) natalensis ♂ M17 from the southern Transvaal.	29
Fig. 10.	Karyotype of Praomys (Mastomys) natalensis ♀ M22 from the southern Transvaal.	30
Fig. 11.	Karyotype of Praomys (Mastomys) natalensis ♂ M60 from the eastern Transvaal.	32
Fig. 12.	Karyotype of Praomys (Mastomys) natalensis ♀ M50 from the eastern Transvaal.	33
Fig. 13.	Karyotype of Praomys (Mastomys) natalensis ♂ M65 from the northern Transvaal.	35
Fig. 14.	Karyotype of Praomys (Mastomys) natalensis ♀ M69 from the northern Transvaal.	36
Fig. 15.	Karyotype of Praomys (Mastomys) natalensis ♂ M20 from South West Africa.	38
Fig. 16.	Karyotype of Praomys (Mastomys) natalensis ♀ M25 from South West Africa.	39
Fig. 17.	Karyotype of Praomys (Mastomys) natalensis ♂ M32 from Rhodesia.	41
Fig. 18.	Karyotype of Praomys (Mastomys) natalensis ♀ M36 from Rhodesia.	42

PAGE

Fig. 19. Karyotype of 31 chromosome cell line in Praomys (Mastomys) natalensis ♂M 31 from Rhodesia. 44

Fig. 2[illegible]. Karyotype of Praomys (Mastomys) natalensis ♀ M 106 from Lesotho. 46

Fig. [illegible]. Map of southern Africa showing collecting sites of Praomys (Mastomys) natalensis investigated in this study. 47

Fig. 22. Metaphase of spermatocyte meiosis in Praomys (Mastomys) natalensis M 12 from the eastern Cape Province showing 18 bivalents. 49

Fig. 23. Metaphase of spermatocyte meiosis in Praomys (Mastomys) natalensis M 17 from the southern Transvaal showing 18 bivalents. 49

Fig. 24. Metaphase of spermatocyte meiosis in Praomys (Mastomys) natalensis M 23 from the northern Cape Province showing 18 bivalents. 50

Fig. 25. Metaphase of spermatocyte meiosis in Praomys (Mastomys) natalensis M 23 from the northern Cape Province showing 18 bivalents. 50

Fig. 26. Metaphase of spermatocyte meiosis in Praomys (Mastomys) natalensis M 23 from the northern Cape Province showing 18 bivalents. 51

Fig. 27. Metaphase of spermatocyte meiosis in Praomys (Mastomys) natalensis M 20 from South West Africa showing 16 bivalents. 53

Fig. 28. Metaphase of spermatocyte meiosis in Praomys (Mastomys) natalensis M 20 from South West Africa showing 16 bivalents. 53

Fig. 29. Metaphase of spermatocyte meiosis in Praomys (Mastomys) natalensis M [illegible] South West Africa showing 16 bivalents. 54

Fig. 30. Metaphase of spermatocyte meiosis in Praomys (Mastomys) natalensis M 30 from South West Africa showing 16 bivalents. 54

Fig. 31. Metaphase of spermatocyte meiosis in Praomys (Mastomys) natalensis M 30 from South West Africa showing 16 bivalents. 55

Fig. 32. Line up of karyotypes of 2n = 36 Praomys (Mastomys) natalensis ♂ from the southern Transvaal and 2n = 32 Praomys (Mastomys) natalensis ♂ from the eastern Transvaal to demonstrate the differences between the karyotypes. 62

PAGE

Fig. 33. Karyotype of a male hybric from a Rhodesia ♂ (2n = 32) X Baragwanath Laboratory Strain ♀ (2n = 36). 73

Fig. 34. Karyotype of a female hybrid from a Rhodesia ♂ (2n = 32) X Baragwanath Laboratory Strain ♀ (2n = 36). 73

Fig. 35. Karyotype of a male hybrid from a Baragwanath Laboratory Strain ♂ (2n = 36) X Rhodesia ♀ (2n = 32). 74

Fig. 36. Karyotype of a female hybrid from a Baragwanath Laboratory Strain ♂ (2n = 36) X Rhodesia ♀ (2n = 32). 74

Fig. 37. Karyotype of a male hybrid from a Baragwanath Laboratory Strain ♂ (2n = 36) X South West Africa ♀ (2n = 32). 76

Fig. 38. Karyotype of a female hybrid from a Baragwanath Laboratory Strain ♂ (2n = 36) X South West Africa ♀ (2n = 32). 76

Fig. 39. Karyotype of a male hybrid from a South West Africa ♂ (2n = 32) X Baragwanath Laboratory Strain ♀ (2n = 36). 77

Fig. 40. Karyotype of a female hybrid from a South West Africa ♂ (2n = 32) X Baragwanath Laboratory Strain ♀ (2n = 36). 77

Fig. 41. Section through testis of a normal breeding ♂ Mastomys M95 of Baragwanath Laboratory Strain. 80

Fig. 42. Section through testis of hybrid ♂ M73 from cross between Baragwanath Laboratory Strain ♂ X South West Africa ♀ Mastomys. 80

Fig. 43. Section through testis of hybrid ♂ M83 from cross between Baragwanath Laboratory Strain ♀ X South West Africa ♂ Mastomys. 81

Fig. 44. Higher magnification of a portion of a tubule in the testis of normal ♂ Mastomys M95 in Fig. 41. .. 81

Fig. 45. Higher magnification of a portion of a tubule in the testis of hybrid ♂ M73 in Fig. 42. 82

Fig. 46. Higher magnification of a portion of a tubule in the testis of hybrid ♂ M83 in Fig. 43. 82

Fig. 47. Section through the epididymis of normal ♂ Mastomys M95 showing tubules filled with spermatozoa. .. 83

PAGE

Fig. 48. Section through the epididymis of hybrid ♂ M83 showing small empty tubules surrounded by interstitial connective tissue. 83

Fig. 49. Section through ovary of a normal mature Baragwanath Laboratory Strain Mastomys ♀ M96. .. 86

Fig. 50. Section through ovary of hybrid ♀ M86 from cross between Baragwanath Laboratory Strain ♂ X South West Africa ♀ Mastomys. 86

Fig. 51. Section through ovary of hybrid ♀ M79 from cross between Baragwanath Laboratory Strain ♂ X South West Africa ♀ Mastomys. 87

Fig. 52. Section through ovary of hybrid ♀ M84 from cross between Baragwanath Laboratory Strain ♂ X South West Africa ♀ Mastomys. 87

Fig. 53. Diagram of possible phylogenetic relationship and chromosome rearrangements in 2n = 32 and 2n = 36 Mastomys. 106

Fig. 54. Provisional geographic distribution of the three forms of Mastomys (from Matthey, 1966a). 108

Fig. 55. Provisional geographic distribution of the three forms of Mastomys, showing new range of distribution in southern Africa resulting from the present investigation. 108

LIST OF TABLES

PAGE

Table 1. Number and sex of animals from each area investigated. 8

Table 2. Results of chromosome counts in Praomys (Mastomys) natalensis from the eastern Cape Province. .. 18

Table 3. Results of chromosome counts in Praomys (Mastomys) natalensis from the northern Cape Province. .. 19

Table 4. Results of chromosome counts in Praomys (Mastomys) natalensis from the northern Orange Free State. .. 22

Table 5. Results of chromosome counts in Praomys (Mastomys) natalensis from the southern Transvaal. 25

Table 6. Results of chromosome counts in Praomys (Mastomys) natalensis from the eastern Transvaal. 28

Table 7. Results of chromosome counts in Praomys (Mastomys) natalensis from the northern Transvaal. 34

Table 8. Results of chromosome counts in Praomys (Mastomys) natalensis from South West Africa. 37

Table 9. Results of chromosome counts in Praomys (Mastomys) natalensis from Rhodesia. 40

Table 10. Results of chromosome counts in Praomys (Mastomys) natalensis from Lesotho. 43

Table 11. Summary of results of chromosome counts and morphological divisions of chromosomes from the Praomys (Mastomys) natalensis specimens obtained in southern Africa. 56

Table 12. Chromosome counts of hybrids obtained from Baragwanath Laboratory Strain (Bara. L. S.) 2n = 36 crossed with Rhodesian (Rhod.) or South West African (S.W.A.) Mastomys 2n = 32. 71

Table 13. Morphological division of hybrid chromosome complement with arrows indicating those chromosomes contributed by the haploid gametes of each parent. 72

Table 14. Morphological division of hybrid chromosome complement with arrows indicating those chromosomes contributed by the haploid gametes of each parent. 75

Table 15. Comparison of paired testes weights of normal breeding Mastomys ♂'s and hybrid Mastomys ♂'s. 78

Table 16. Comparison of the weights of ovaries and uteri in normal breeding Mastomys ♀'s and hybrid Mastomys ♀'s. 85

PAGE

Table 17. A comparison of chromosome types and N.F. in Praomys (Mastomys) natalensis with 32 chromosomes from different areas in sub-Saharan Africa. .. 99

Table 18. A comparison of chromosome types and N.F. in Praomys (Mastomys) natalensis with 36 chromosomes from different areas in southern Africa. 101

Table 19. A comparison of chromosome types and N.F. in Praomys (Mastomys) erythroleucus with 38 chromosomes from West Africa. 103



FRONTISPIECE. Praomys (Mastomys) natalensis

CHAPTER 1

INTRODUCTION

The species is the basic unit of biological classification and as such is of great importance in many branches of natural science. Mayr (1963) defined a species as a group of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups. The formation of new species is one of the most important events in evolution and many biologists and biochemists study systems and compounds which are species specific. In the fields of medical entomology, parasitology and zoonotic diseases the ability to identify accurately hosts and vectors has greatly increased the understanding and control of infections communicable to man.

However taxonomists are finding it increasingly difficult to rely only on morphological characteristics for the classification of animals at the species level. They are having to depend more and more on other disciplines to confirm or reject their assumptions. Evol the slow process it is adds to the confusion as many populations may .ly progressed part of the way in the process of species formation. Then there are those populations which have achieved reproductive isolation from morphologically similar populations. These are known as sibling species and can only be recognised by methods other than appearance.

Since the development of reliable techniques for estimating chromosome numbers in animals, cytogeneticists have been able to be of some assistance

in this classification problem. This was demonstrated in the situation which arose in Iranian Kurdistan. Plague investigations were hampered until it was established by cytogenetical examination that there were four separate species of the gerbil Meriones in the area and not two as previously thought. It was then established that there were two susceptible and two resistant species involved in the plague cycle (Baltazard, 1963).

The multimammate mouse is a semi-domestic rodent of the family Muridae and is one of those species which on cytological evidence has been shown not to be the simple uniform group it appeared to be on morphological characters. According to Ellerman, Morrison-Scott and Hayman (1953) its range extends from a southern limit in the eastern Cape Province throughout Africa south of the Sahara to a northern limit in Morocco in the far north west. Because of its semi-domestic nature it has been suggested that this distribution is dependent on having followed early human population movements (Davis, 1962).

The nomenclature of the multimammate mouse has received considerable attention. It was known as Mastomys coucha until Roberts (1944) pointed out that this name had been used for a mouse which in an earlier description had been called Mastomys natalensis. The genus Mastomys was relegated to a subgenus of Rattus (Ellerman, 1941, Ellerman, Morrison-Scott and Hayman, 1953) and for a considerable period of time the multimammate mouse was Rattus (Mastomys) natalensis. Though Mastomys was given generic status again by various authors (Lundholm, 1955, Setzer, 1956), Davis in 1962 used Praomys as the generic name for the South African species of natalensis. The current valid name is Praomys (Mastomys) natalensis as used in the

provisional key of Davis (1965), who used the alveolar root pattern as the basis for his classification, and in the Smithsonian Institution preliminary identification Manual for African Mammals (Misonne, 1968).

As it is a multimammate mouse it has large litters of anything from twelve to twenty (Roberts, 1951). It is well developed as a laboratory animal and because it such a productive species is widely used as an experimental animal, particularly in the fields of plague, bilharziasis and cancer (Davis and Oettlé, 1958, Davis, 1963). It has thus come to the attention of cytologists.

The Praomys (Mastomys) natalensis occurring in South Africa were investigated by Matthey (1954), who found them to have 36 chromosomes or 18 pairs. This finding was confirmed by Huang and Strong in 1962 using mice from the same source as those used by Matthey in 1954, namely a colony of laboratory bred mice housed at the South African Institute for Medical Research in Johannesburg. This colony was bred from mice originally caught at Baragwanath, sixteen kilometres south west of Johannesburg. It is possible that despite precautions, some interbreeding of these mice with an old stock from the Transkei and the Rooiwal area of the Orange Free State did take place (Davis and Oettlé, 1958).

Matthey (1955) also examined some Praomys (Mastomys) natalensis from Oubanghi in the Central African Republic and found these to have 32 chromosomes or 16 pairs, two pairs less than the South African Mastomys. Matthey (1955) thought this reduction in chromosome number could partly be the result of simple Robertsonian fusion between two pairs of chromosomes, but techniques in use then were not sufficient to give clear karyotypes, so

this was not substantiated. Because of the variation between the chromosomes of these mice from the Central African Republic and those from South Africa, Matthey suggested these forms be considered as separate species. Breeding experiments conducted between mice of these two chromosome groups at the Medical Ecology Centre, Johannesburg produced hybrids with 34 chromosomes (Dickson, 1967).

Another investigation by Matthey in 1958 of the chromosomes of Mastomys natalensis erythroleucus from the Ivory Coast showed these mice to have a complement of 40 chromosomes. This led him to agree with a classification by Petter (1957) that 'erythroleucus' was a 'good' species and not a subspecies of natalensis.

Praomys (Mastomys) natalensis from Fort Lamy in Chad also have 32 chromosomes (Matthey, 1966 b). Using the recommended technique of colchicine injection into specimens before chromosome analysis (Ford and Hamerton, 1956a), Matthey (1966 a) again looked at the chromosomes of Praomys (Mastomys) natalensis from the Ivory Coast, the Central African Republic, South Africa and in addition some from Congo-Brazzaville. The previous observations were confirmed except that Praomys (Mastomys) erythroleucus was found to have 38 chromosomes and not 40 as previously described. Those from the Congo-Brazzaville had 32 chromosomes. He was also able to confirm that centric fusions have played an important part in Praomys (Mastomys) evolution. Matthey (1966 a) re-affirmed that the species Praomys (Mastomys) natalensis therefore brings together under the same denomination several forms which have such different chromosome

complements that they would no longer be able to interbreed and should be considered as separate species. Two of these forms are sympatric in West Africa, the one with 32 and the other with 38 chromosomes. Those with 38 appear to be Praomys (Mastomys) erythroleucus, which is considered a true species and not a form of natalensis. In South Africa only a form with a diploid number of 36 has been found. How far south the 32 chromosome species extends and how far north the 36 chromosome form extends has yet to be determined.

However the Praomys (Mastomys) natalensis in South Africa have not been very thoroughly investigated. The chromosomes of a number of specimens all originating from the same area near Johannesburg, have been counted (Matthey, 1954, 1966a, Huang and Strong, 1962), but this animal is widely distributed in southern Africa. It was therefore felt that a cytogenetic investigation of the multimammate mice occurring in southern Africa was necessary. Southern Africa in this investigation is defined as that part of Africa south of the Cunene and Zambesi Rivers. Figure 1 is a map of this area showing the recorded distribution of Praomys (Mastomys) natalensis compiled by the Medical Ecology Centre, Johannesburg and includes records up until 1973.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

Multimammate mice are classified as follows:

Order	:	Rodentia
Sub-Order	:	Myomorpha
Family	:	Muridae
Sub-Family	:	Murinae
Genus	:	Praomys
Sub-Genus	:	Mastomys
Species	:	natalensis

2.1.1 Localities investigated

Animals of the species, Praomys (Mastomys) natalensis, were obtained from as many different parts of southern Africa as possible. The mice were caught by hand in many instances, or otherwise in baited traps, the most common of these being the Sherman trap. The Technical Field Staff of the State Department of Health kindly provided many of the animals and others were obtained through personal contacts. From these sources mice were obtained from nine areas within southern Africa as follows:

(i) the Port Elizabeth area of the eastern Cape Province

(ii) Vryburg and Kuruman in the northern Cape Province

(iii) Kroonstad and Heilbron in the northern Orange Free State

(iv) Benoni and Johannesburg areas of the southern Transvaal

(v) Kaapmuiden area of the eastern Transvaal

(vi) Tzaneen area of the northern Transvaal

(vii) Windhoek, South West Africa

(viii) Salisbury, Rhodesia

(ix) Roma, Lesotho

The number and sex of the mice from each of the areas are given in Table 1.

Table 1. Number and sex of animals from each area investigated.

Area	Males	Females	Total
Eastern Cape Province	6	3	9
Northern Cape Province	1	3	4
Northern Orange Free State	3	2	5
Southern Transvaal	1	3	4
Eastern Transvaal	7	7	14
Northern Transvaal	3	3	6
South West Africa	3	2	5
Rhodesia	3	6	9
Lesotho	2	1	3
Total	29	30	59

The mice were identified as Praomys (Mastomys) natalensis by staff of the Medical Ecology Centre, Johannesburg. Note was taken of the number of mammae in the females as Praomys (Mastomys) shortridgei, found in the Okavango area of South West Africa, has only 10 mammae whereas Praomys (Mastomys) natalensis has 12 or more.

The collecting sites were plotted on a 1 : 5 000 000 scale map using the quarter-degree square method of mapping (Davis, 1948). The map is printed in black and has a superimposed blue grid divided into quarter-degree squares. This is the same scale map as used by the Medical Ecology Centre for plotting the general distribution of Praomys (Mastomys) natalensis in Chapter 1 (Figure 1). The quarter-degree square method of mapping involves the dividing of a latitude and longitude degree square into four, and each of these four half-degree squares is divided into four again to give quarter-degree squares. The quarter-degree square is referred to by a code, which is the latitude and longitude followed by the half-degree square symbol and thereafter by the quarter-degree square symbol. Figure 2 illustrates the quarter-degree square system of mapping for Kuruman, the co-ordinates of which are 27° 27' South latitude and 23° 26' East longitude.

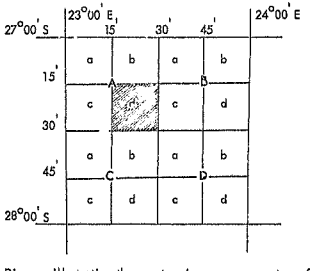


Fig. 2. Diagram illustrating the quarter-degree square system of mapping. Kuruman is indicated by the code 2723 Ad which is the shaded square.

2.2 Methods

2.2.1 Brief background to techniques in use for chromosome study from bone marrow of small mammals

The Feulgen squash technique, originally devised for the study of plant chromosomes in the 1930's, was the first in a series of developments which led to the techniques now used for the study of mammalian chromosomes. Early improvements in the squash technique were firstly the use of acetic-alcohol fixation (Sachs, 1952) which meant that one of the least hardening fixatives was being used, and secondly water pretreatment of the chromosomes (Makino and Nishimura, 1952). This pretreatment of the chromosomes with a hypotonic solution was also advocated by Hughes (1952). He found that treatment of dividing cells in chick tissue culture with hypotonic culture medium caused the cells to swell, which improved chromosome dispersion and also resulted in slight shortening and spreading of the chromatids. Hsu (1952) found the same effects using hypotonic culture medium on human fibroblasts. Since then many different hypotonic agents have been used, but Ford (1973) suggested that in the absence of controlled trials any reasonably physiological substance of the right tonicity used for a given time would give satisfactory results.

For the examination of small mammal chromosomes Ford and Hamerton (1956a) recommended intraperitoneal injection of colchicine about one hour prior to their being killed. Colchicine and other spindle inhibiting agents have long been used in plant cytological techniques to improve chromosome dispersion. Ford and Hamerton (1956a) used the techniques of colchicine injection and hypotonic treatment in conjunction and found them to complement one another.

The colchicine shortened the chromosomes and caused the sister chromatids to diverge, while the hypotonic pretreatment expanded the cells. They also advocated working with cell suspensions where possible to allow even penetration of fixative and other reagents.

Rothfels and Siminovitch (1958) then introduced a method of obtaining well-spread metaphase plates without recourse to manual squashing, but incorporating other recently developed techniques. The essential part of their treatment was the complete air drying of films of cells following fixation. The appearance of the chromosomes was not distorted and an even and complete flattening of dividing nuclei without chromosome scattering was obtained. Crowding of cells on the slide had to be avoided.

2.2.2 Cytological techniques

The procedures used in this investigation are described below. These combine the colchicine hypotonic treatment recommended by Ford and Hamerton (1956a) and the air drying method of obtaining metaphase plates described by Rothfels and Siminovitch (1958).

(i) The mice were injected intraperitoneally with 0.5 cc of a 0.05 percent solution of colchicine. This was the amount recommended by Ford and Hamerton (1956a), adjusted for the weight of an average adult Praomys (Mastomys) natalensis, which was about 50 g. Younger mice were given proportionally less colchicine. The mice were then left for 1½ hours minimum to 2 hours maximum before being sacrificed. Leaving the colchicine in the mice for longer periods than this caused clumping of the chromosomes (Ford and Hamerton, 1956a).

(ii) The mice were killed with ether. Immediately after death the femora were rapidly dissected out, the epiphyses removed and the bone marrow washed from the shafts with warm culture medium (M 150) using a hypodermic syringe. As the time in hypotonic solution was fairly critical, it was better to use culture medium for the removal of the bone marrow and not the hypotonic solution itself as used by Ford and Hamerton (1956a) Aspiration of the bone marrow in and out of the syringe broke up the marrow into a suspension of cells. No anticoagulant was necessary with the small amount of bone marrow obtained from the femora. Where the specimen was fairly small, bone marrow was taken from the tibias as well. Bone marrow was used as it had a high percentage of young, actively dividing cells and thus the number of cells arrested at metaphase would form a fairly high percentage of the total cells harvested.

(iii) The suspension was then transferred to a 10 cc graduated centrifuge tube and centrifuged at 800 r.p.m. for 5 minutes. The supernatant fluid was discarded by gently tilting the centrifuge tube and pouring off the supernatant. Care was taken not to disturb the button of cells at the base of the tube.

(iv) The cells were suspended in 5.0 cc of a hypotonic solution of 0.075M potassium chloride and left for 2 minutes. Potassium chloride was first recommended for use in hypotonic treatment by Hungerford in 1965. He found the effects of the potassium chloride to be much less extreme than other hypotonic agents such as the sodium citrate used by Ford and Hamerton (1956a), or Hanks balanced salt solution (Moorehead et al, 1960). The potassium chloride was their equivalent in the efficiency with which it

disrupted spindle organization, but permitted the chromosomes in metaphase plates to become well separated from one another.

(v) The suspension was then centrifuged for 5 minutes, so that the cells were in the hypotonic solution for a maximum of 7 minutes, including spinning time. Hungerford (1965) recommended 10 minutes for human leucocytes, but 7 minutes was found to be sufficient for Mastomys bone marrow cells. A longer time caused excess swelling of the cell. This seemed to result in the cell breakage during slide preparation and the chromosomes being scattered too far and wide to be of any use in chromosome enumeration. The supernatant was discarded in the manner described above.

(vi) The cells were then fixed with a 3 : 1 absolute methyl alcohol : glacial acetic acid fixative. At the start this was done by adding the fixative dropwise into the centrifuge tube, while the button of cells in the bottom of the tube was being agitated. This dropwise addition of the fixative ensured a fine suspension of cells. Thereafter the fixative was added more rapidly to the 5.0 cc mark of the centrifuge tube. The cells were left in this fixative for 10 minutes.

(vii) The cell suspension was centrifuged again at 800 r.p.m. for 5 minutes and the supernatant discarded.

(viii) Freshly made 3 : 1 absolute methyl alcohol : glacial acetic acid fixative was then added to the cells in the tube, and the button of cells was broken up to disperse the cells in the fixative. Freshly made fixative must be used because methyl acetate forms rapidly and this has been found to interfere with good fixation. The cells were left in this fixative for a minimum of 20 minutes to ensure thorough penetration of the fixative.

(ix) After this long fixation the suspension was again centrifuged at 800 r.p.m., the supernatant poured off and a few drops of fresh fixative added to the button of cells, enough to make a cloudy suspension. This usually provided the right dilution to prepare the slides although further dilution was necessary when the percentage of mitotic activity in the cells was high.

(x) Fresh fixative was allowed to run over clean slides tilted at a slight angle. Immediately following this a few drops of the cell suspension were added to the slide and allowed to flow down the slide. The slide was then waved quickly in the air to allow rapid drying of the cells and to ensure spreading of the metaphase chromosomes. This method of preparation provided the best spreading of the chromosomes with the least damage to them and proved to be a very simple and quick method to use when numerous slides had to be prepared.

(xi) The slides were stained according to Jacobsen's method, a combination stain consisting of May Grunewald and Giemsa (Hsu, 1952). Stock May Grunewald was made by dissolving 2.5 g of the May Grunewald powder in 1 000 cc methanol. This was heated slightly, stirred and then filtered. Stock Giemsa was made by dissolving 1.0 g of the Giemsa powder in 66 cc extra pure glycerine at 60°C, while stirring. When cool this was mixed with 60 cc methanol and filtered.

The staining procedure was as follows:

(a) Slides were fixed in methanol for 15 minutes.

(b) The slides were removed from the methanol and air dried.

(c) When dry, the slides were rinsed in phosphate buffer pH 6.7.

(d) Slides were stained in May Grunewald for 20 to 30 minutes (stock solution was diluted 1 : 10 with buffer pH 6.7).

(e) Slides were rinsed in buffer pH 6.7.

(f) Slides were stained in Giemsa for 20 minutes (stock solution was diluted 1 : 20 with buffer pH 6.7).

(g) The slides were rinsed in buffer pH 6.7, care being taken not to remove too much of the stain.

(h) The slides were air dried.

(i) When completely dry the slides were mounted in D.P.X.

This staining procedure gave the greatest clarity to the chromosomes and appeared to be more permanent than many other chromosome stains.

2.2.3 Karyotyping methods

The slides were scanned systematically under the microscope using a 10X eyepiece and 10X objective. Clear metaphase plates with very few or no overlapping chromosomes were selected for counting at this 100X magnification. The selected plates were then counted under 1 000X magnification (oil immersion) regardless of their appearance at this higher magnification so that no personal bias was introduced. Thirty plates from each mouse were counted as this was thought to be a sufficient number of cells to detect any mosaicism within a specimen. It was possible to count thirty plates from all the specimens, except three from the eastern Transvaal. Six plates were counted in one of these specimens, but none could be counted in the other two specimens. This was found to be due to contaminated alcohol used in the fixative. Plates with very clear and no overlapping chromosomes were photographed for karyotyping.

Photographs of the metaphase plates were taken using a Wild Automat 35mm camera on a Reichert Bizet microscope with 100X oil immersion objective and 10X eyepiece. On some occasions a 6X eyepiece was used. The film used was Kodak microfile which was developed with Kodak D76 for 10 minutes at 20°C, washed, then fixed in May and Baker Amfix for 2 minutes. The photomicrographs were printed on Kodak 3 or 4 paper using Kodak developer 163 in a 1 in 3 dilution.

Chromosomes from the photomicrographs were carefully cut out and the karyotypes of the mice from the different geographical areas constructed. The chromosomes were arranged in seemingly homologous pairs and grouped according to size and the position of the centromeres, namely into submetacentric, metacentric and acrocentric groups. As some of the chromosomes had one pair of very short arms their placing in either the submetacentric or acrocentric groups was somewhat arbitrary. The X and Y chromosomes were grouped separately.

2.2.4 Investigations of meiotic activity

The cauda epididymidis was macerated in normal saline and examined microscopically for the presence of spermia. Meiotic activity within the testes was investigated by making cell suspensions of testicular material in isotonic sodium citrate (2.2 percent wt./vol.) at room temperature after the method of Evans, Breckon and Ford (1964). Thereafter the cell suspension was subject to the same hypotonic centrifuging and fixing procedure as for bone marrow. The slides were also made and stained in the same manner as for bone marrow. Clendenin (1969) used hamsters pretreated with colchicine for one

or two hours for meiotic studies and these showed a markedly increased yield of cells in first meiotic metaphase in comparison with animals with no pretreatment. For this reason the mice injected with colchicine for mitotic studies on bone marrow were used for establishing meiotic activity in this investigation, as the number of mice available was limited. Clendenin (1969) also found that hypotonic potassium chloride resulted in much sharper chromosome definition than 1.1 percent sodium citrate which is widely used in meiotic studies. Meiotic activity was examined in fixed and stained sections as well.

2.2.5 Histological techniques

For histological examination the reproductive organs were dissected from mice which had not been pretreated with colchicine. The paired testes or ovaries plus uteri were then weighed on a torsion balance and transferred immediately to Bouin's solution. The tissues were dehydrated in an Elliot Tissue Processor and wax blocks prepared. Sections of 4 micra thickness were cut and stained with haematoxylin and eosin.

CHAPTER 3

RESULTS OF CHROMOSOME STUDIES IN PRAOMYS (MASTOMYS) NATALENSIS IN SOUTHERN AFRICA

3.1 Mitotic Studies

The chromosomes of the mice from each area are described under separate headings below. The quarter-degree square from which the specimens came is marked on a map of southern Africa, Figure 21, which appears at the end of the descriptions of the mitotic chromosomes in this chapter (see Page 47).

3.1.1 Specimens from the eastern Cape Province

Six male and three female specimens of Praomys (Mastomys) natalensis were obtained from Addo near Port Elizabeth (map reference 3325 Ad). Chromosome counts from mitotic metaphase plates, obtained from bone marrow suspensions of these animals, are given in Table 2.

Table 2. Results of chromosome counts in Praomys (Mastomys) natalensis from the eastern Cape Province.

Mouse number	Sex	Chromosome number Number of cells counted with	
		<36	36
M3	♂	1	29
M7	♂		30
M8	♀		30
M9	♀		30
M12	♂		30
M13	♂		30
M14	♂	2	28
M15	♀		30
M16	♂	1	29
Total	9	4	266

Chromosome counts revealed the number to be 36 in all but 4 of 270 cells counted. Karyotypes constructed from photomicrographs of metaphase plates showed:-

6 pairs of submetacentric chromosomes

5 pairs of metacentric chromosomes

6 pairs of acrocentric chromosomes

plus the two sex chromosomes. In the female the X chromosomes were the two largest metacentric chromosomes in the karyotype, and the Y in the male was a large submetacentric chromosome similar to the largest pairs of submetacentric autosomes. *Karyotypes of a male (M7) and a female (M8)* are given in Figures 3 and 4.

3.1.2 Specimens from the northern Cape Province

Only 4 mice were obtained from this area, one male (M23) and one female (M24) from a farm near Kuruman (map reference 2723 Ad) and two females, M4 and M59, from Vryburg (map reference 2624 Dc). Table 3 gives the chromosome counts from these mice.

Table 3. Results of chromosome counts in Praomys (Mastomys) natalensis from the northern Cape Province.

Mouse number	Sex	Chromosome number Number of cells counted with	
		< 36	36
M4	♀	1	29
M23	♂		30
M24	♀		30
M59	♀		30
Total	4	1	119

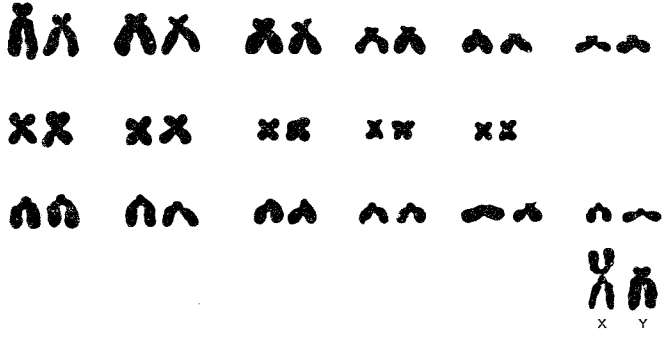


Fig. 3. Karyotype of Praomys (Mastomys) natalensis ♂ M7 from the eastern Cape Province.

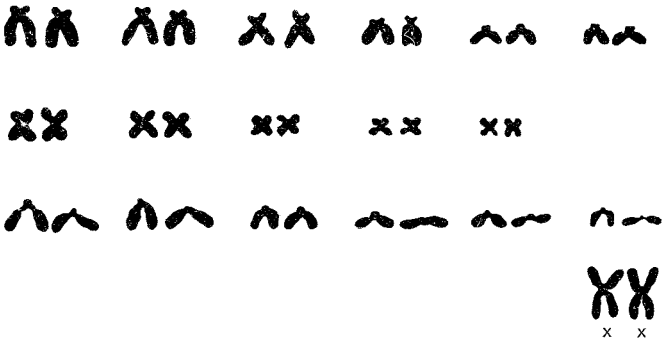


Fig. 4. Karyotype of Praomys (Mastomys) natalensis ♀ M8 from the eastern Cape Province.

The modal chromosome count of these mice was 36, similar to those from the eastern Cape Province. From karyotypes constructed from metaphase plates the morphological appearance of the chromosomes was as follows:-

6 pairs of submetacentric chromosomes

4 pairs of metacentric chromosomes

7 pairs of acrocentric chromosomes

plus the sex chromosomes. The X chromosomes were very large metacentric chromosomes and the Y was a large submetacentric chromosome. Figures 5 and 6 show the karyotype of a male (M23) and a female (M24) from this area. These mice differed from the eastern Cape Province mice in having only 4 pairs of metacentric chromosomes and one extra pair of chromosomes, most probably in the acrocentric group.

3.1.3 Specimens from the northern Orange Free State

Five mice were collected from this area, 2 males and 2 females from Heilbron (map reference 2727 Bd) and one male from Kroonstad (map reference 2727 Cb). A modal count of 36 chromosomes was obtained from the bone marrow suspensions of these mice as shown in Table 4.

Table 4. Results of chromosome counts in Praomys (Mastomys) natalensis from the northern Orange Free State.

Mouse number	Sex	Chromosome number Number of cells counted with	
		< 36	36
M58	♂		30
M61	♀	1	29
M62	♂		30
M63	♂	?	28
M64	♀		30
Total	5	3	147

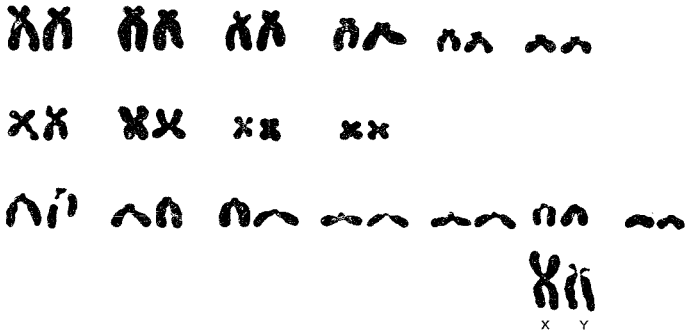


Fig. 5. Karyotype of Praomys (Mastomys) natalensis ♂ M23 from the northern Cape Province.

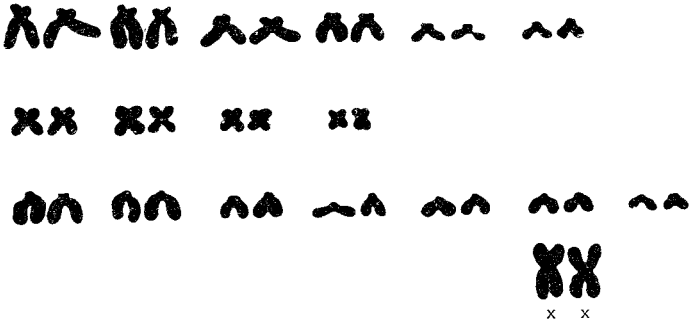


Fig. 6. Karyotype of Praomys (Mastomys) natalensis ♀ M24 from the northern Cape Province.

The karyotypes of these Mastomys showed the distribution of chromosome types to be the same as that described for the mice from the eastern Cape Province, viz. :-

6 pairs of submetacentric chromosomes

5 pairs of metacentric chromosomes

6 pairs of acrocentric chromosomes

plus the two sex chromosomes. The sex chromosomes were also the same, the X being a very large metacentric and the Y one of the largest submetacentric chromosomes. Examples of these karyotypes are shown in Figures 7 and 8.

3.1.4 Specimens from the southern Transvaal

Four mice came from this area, one male and two females from a farm "Vlakfontein", just outside Johannesburg (map reference 2627 Bb) while one female, M 51 was collected 16 kilometres from Benoni (map reference 2628 Ab). Table 5 gives the chromosome counts of these mice.

Table 5. Results of chromosome counts in Praomys (Mastomys) natalensis from the southern Transvaal.

Mouse number	Sex	Chromosome number Number of cells counted with	
		< 36	36
M 17	♂	1	29
M 18	♀		30
M 22	♀		30
M 51	♀		30
Total	4	1	119

These mice had a modal chromosome count of 36 and a karyotype pattern of:



Fig. 7. Karyotype of Praomys (Mastomys) natalensis ♂ M62 from the northern Orange Free State.

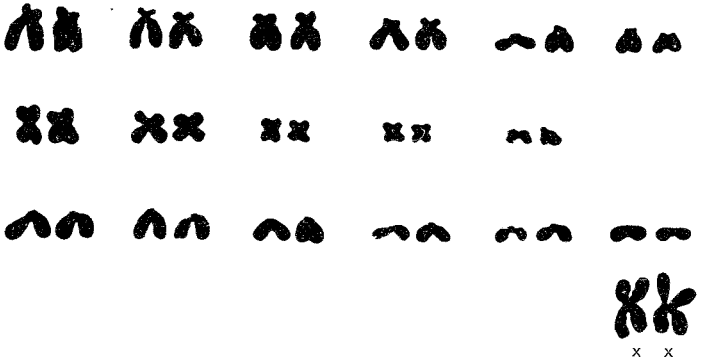


Fig. 8. Karyotype of Praomys (Mastomys) natalensis ♀ M64 from the northern Orange Free State.

6 pairs of submetacentric chromosomes

5 pairs of metacentric chromosomes

6 pairs of acrocentric chromosomes

and the two large sex chromosomes (see Figures 9 and 10 for examples of this karyotype). This was a similar karyotype to that of the eastern Cape Province and northern Orange Free State specimens studied.

3.1.5 Specimens from the eastern Transvaal

Seven males and six females were obtained from a farm " Schoemansdal" near Kaapmuiden (map reference 2531 Cb). The chromosome counts for these mice are given in Table 6.

Table 6. Results of chromosome counts in Praomys (Mastomys) natalensis from the eastern Transvaal.

Mouse number	Sex	Chromosome number Number of cells counted with	
		<32	32
M 37	♂	1	29
M 38	♂		30
M 39	♂	1	29
M 40	♀		30
M 42	♀		6
M 43	♀	1	29
M 44	♀		30
M 46	♀		-
M 47	♀		-
M 48	♂		30
M 49	♂	1	29
M 50	♀		30
M 60	♂		30
Total	13	4	302

Some of the chromosome preparations from these mice failed completely, while others gave rather poor quality metaphase plates. When they failed completely it was possible that the colchicine was too old to be effective,

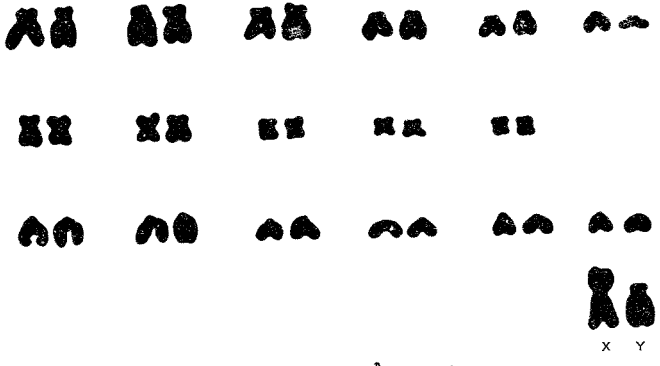


Fig. 9. Karyotype of Praomys (Mastomys) natalensis ♂ M17 from the southern Transvaal.

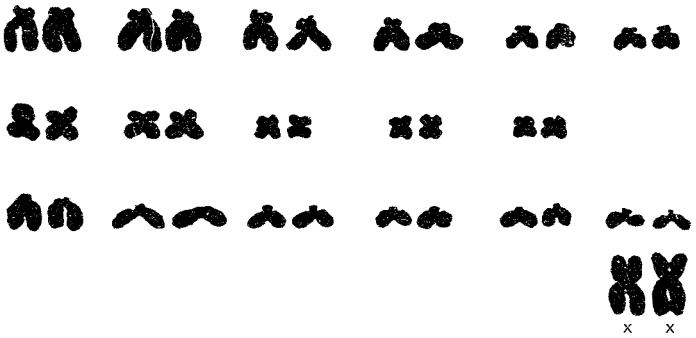


Fig. 10. Karyotype of Praomys (Mastomys) natalensis ♀ M22 from the southern Transvaal.

while those of poor quality indicated bad fixation, probably due to contaminated methanol. After these difficulties were overcome the problems were not encountered again. Fortunately there were still sufficient mice from this area with satisfactory metaphase plates so that karyotypes could be constructed.

As Table 6 indicates, the modal chromosome count for these mice was 32 and not 36 as in samples from all the other areas investigated in South Africa so far.

Figures 11 and 12 show representative karyotypes of a male (M60) and a female (M50) from this area. In these mice there were:-

7 pairs of submetacentric chromosomes

5 pairs of metacentric chromosomes

3 pairs of acrocentric chromosomes

plus the two sex chromosomes. The X chromosomes were again the largest metacentric chromosomes in the karyotype. The Y chromosome in these mice was not a submetacentric chromosome morphologically similar to the larger submetacentric chromosomes, as was the case in the mice from the four areas previously described in this chapter. In these mice the Y was the largest single acrocentric chromosome in the karyotype.

3.1.6 Specimens from the northern Transvaal

Three male and one female Praomys (Mastomys) natalensis were sent from a farm, "Calais", near Tzaneen (map reference 2430 Ab) and one female from Magoebaskloof (map reference 2330 Cc). The chromosome counts of these mice are given in Table 7.

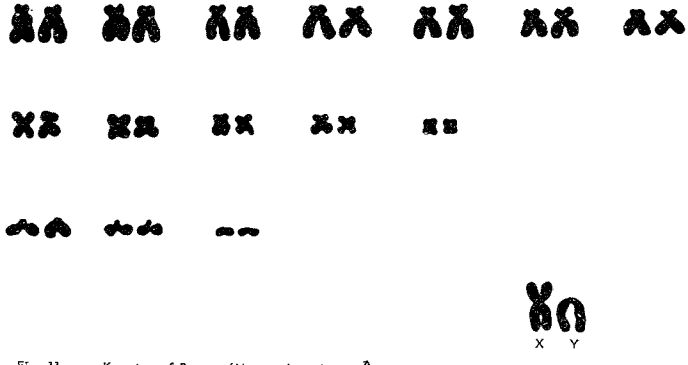


Fig. 11. Karyotype of Praomys (Mastomys) natalensis ♂ M60 from the eastern Transvaal.

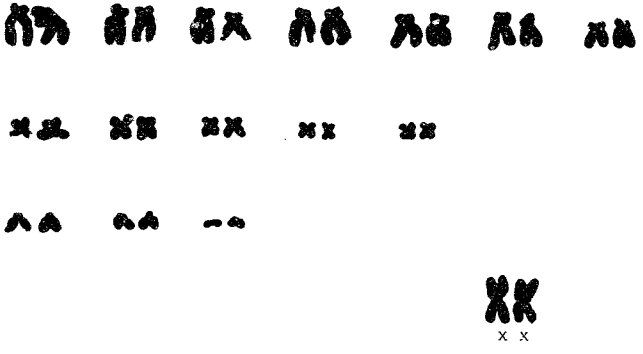


Fig. 12. Karyotype of Praomys (Mastomys) natalensis ♀ M 50 from the eastern Transvaal.

Table 7. Results of chromosome counts in Praomys (Mastomys) natalensis from the northern Transvaal.

Mouse number	Sex	Chromosome number Number of cells counted with <32	32
M65	♂		30
M66	♂	1	29
M68	♂		30
M69	♀	1	29
M70	♀		30
Total	5	2	148

These mice had the same chromosome number as those from the eastern Transvaal. Karyotypes of a male (M65) and a female (M69) in Figures 13 a[illegible] show the morphological groups of chromosomes to be the same as for the eastern Transvaal mice, namely:-

7 pairs of submetacentric chromosomes

5 pairs of [illegible]centric chromosomes

3 pairs of acrocentric chromosomes

plus the sex chromosomes. The Y chromosome was again the largest acrocentric chromosome, whilst the X chromosomes were two large metacentric chromosomes in the female.

3.1.7 Specimens from South West Africa

The three male and two female Praomys (Mastomys) natalensis came from the Windhoek area of South West Africa (map reference 2217 Ca). As these mice came from territory close to that inhabited by Praomys (Mastomys) shortridgei, the identification of these specimens was confirmed by the fact that the females had 16 mammae characteristic of Praomys (Mastomys) natalensis. Chromosome counts of these mice are given in Table 8.

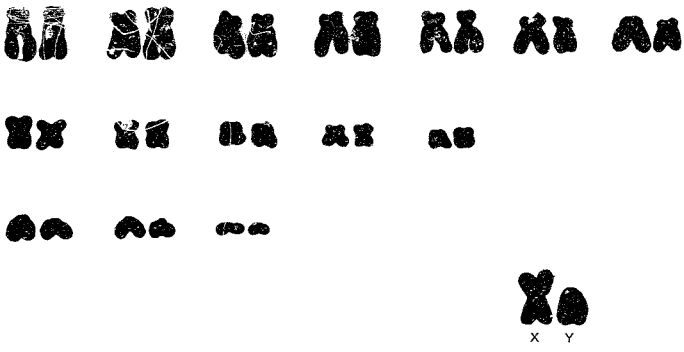


Fig. 13. Karyotype of Praomys (Mastomys) natalensis ♂ M65 from the northern Transvaal.

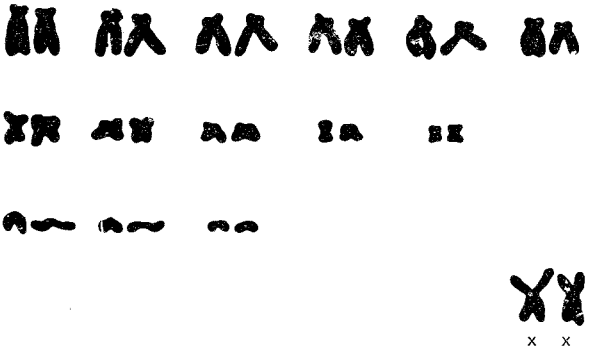


Fig. 14. Karyotype of Praomys (Mastomys) natalensis ♀ M69 from the northern Transvaal.

Table 8. Results of chromosome counts in Praomys (Mastomys) natalensis from South West Africa.

Mouse number	Sex	Chromosome number Number of cells counted with <32	32
M20	♂		30
M25	♀		31
M30	♂		30
M71	♀		30
M72	♂	1	29
Total	5	1	149

These mice had 32 chromosomes or 16 pairs and had therefore a similar chromosome number to the northern and eastern Transvaal groups. Figures 15 and 16 are karyotypes of a male (M20) and a female (M25). The karyotypes showed that the morphological divisions of the chromosomes was similar to that found in the northern and eastern Transvaal specimens, namely:-

7 pairs of submetacentric chromosomes

5 pairs of metacentric chromosomes

3 pairs of acrocentric chromosomes

plus the two sex chromosomes. The Y chromosome was again the largest acrocentric chromosome and the X chromosomes were large metacentric chromosomes.

3.1.8 Specimens from Rhodesia

Three male and six female Praomys (Mastomys) natalensis were received from the Salisbury district of Rhodesia (map reference 1731 Cc). Table 9 gives the chromosome counts for these specimens.

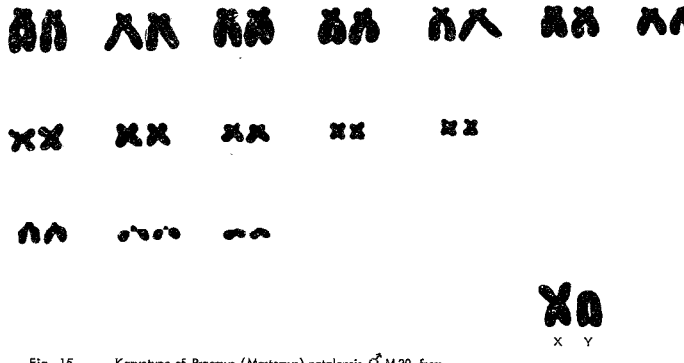


Fig. 15. Karyotype of Praomys (Mastomys) natalensis ♂ M20 from South West Africa.

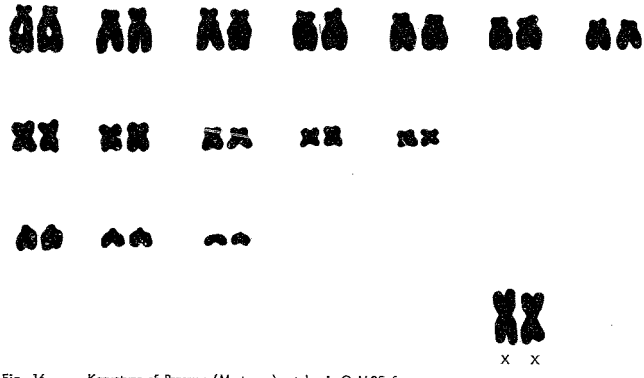


Fig. 16. Karyotype of Praomys (Mastomys) natalensis ♀ M25 from South West Africa.

Table 9. Results of chromosome counts in Praomys (Mastomys) natalensis from Rhodesia.

Mouse number	Sex	Chromosome number Number of cells counted with	
		< 32	32
M 28	♀		30
M 29	♀		30
M 31	♂	17	13
M 32	♂	1	29
M 33	♀		30
M 36	♀		30
M 41	♀		30
M 53	♂		30
M 54	♂		30
Total	9	18	252

A modal chromosome count of 32 was found in 8 out of 9 animals, as in mice from South West Africa, northern and eastern Transvaal. In the 9th specimen from Rhodesia only 31 chromosomes were found in more than half of the cells counted. However, as this was observed in only one mouse, 32 chromosomes must be considered the norm for the present population though the occurrence of a 31/32 animal might suggest a very early stage of a change. Karyotypes of a male (M32) and a female (M36) with 32 chromosomes are given in Figures 17 and 18. From these, it can be seen that the morphological divisions of the chromosomes were slightly different from those in the groups of mice with 32 chromosomes from other areas, and were as follows:-

7 pairs of submetacentric chromosomes

4 pairs of metacentric chromosomes

4 pairs of acrocentric chromosomes

plus the two sex chromosomes. The X chromosomes were large metacentric

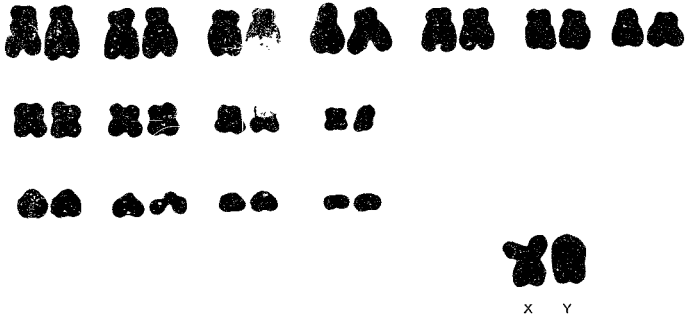


Fig. 17. Karyotype of Praomys (Mastomys) natalensis ♂ M 32 from Rhodesia.

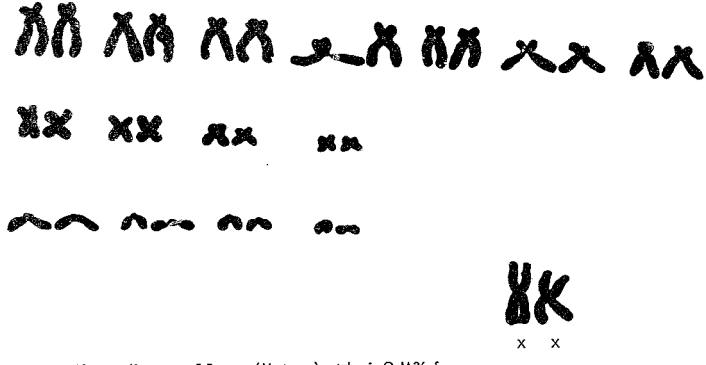


Fig. 18. Karyotype of Praomys (Mastomys) natalensis ♀ M36 from Rhodesia.

chromosomes and the Y chromosome was a large acrocentric chromosome as in the other 32 chromosome groups.

The 31 cell line in the 31/32 mosaic lacked the male acrocentric Y chromosome in all the 31 chromosome metaphase plates examined. Karyotypes constructed of this 31 chromosome cell line showed that this chromosome was missing rather than translocated on to one of the autosomes (Figure 19).

3.1.9 Specimens from Lesotho

Only three mice were obtained from Lesotho; they came from the area around Roma (map reference 2927 Bc). The chromosome counts of the two males and one female are given in Table 10.

Table 10. Results of the chromosome counts in Praomys (Mastomys) natalensis from Lesotho.

Mouse number	Sex	Chromosome number Number of cells counted with	
		< 36	36
M105	♂		30
M106	♀		30
M107	♂		30
Total	3		90

These mice had a modal chromosome count of 36, the same as that found in specimens from the northern and eastern Cape Province, northern Orange Free State and southern Transvaal.

The slides used for bone marrow preparations from these specimens were too thick to allow for good resolution in photography. A karyotype of a female was obtained, though this was not very clear, but no accurate photographs were obtainable from the male metaphase plates. That of the

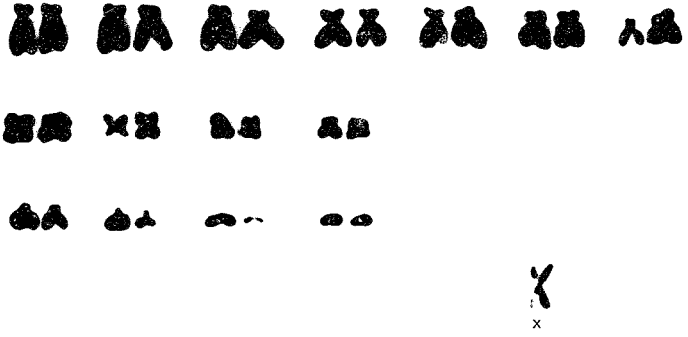


Fig. 19. Karyotype of 31 chromosome cell line in Praomys (Mastomys) natalensis ♂ M31 from Rhodesia.

female (Figure 20) showed the chromosome morphological grouping to be the same as that described for the eastern Cape Province, northern Orange Free State and southern Transvaal specimens, namely:-

6 pairs of submetacentric chromosomes

5 pairs of metacentric chromosomes

6 pairs of acrocentric chromosomes

plus the two large X chromosomes. One of these X chromosomes appeared to be rather more submetacentric than metacentric.

3.2 Meiotic Studies

In most species of mammals the X chromosome is smaller in size than the first pair of autosomes (Vorontsov, 1973). In Mastomys the X is larger than any autosome and the Y is also one of the largest chromosomes in the karyotype. The occurrence of large X chromosomes and sometimes large Y chromosomes in some mammals is of interest to cytotaxonomists. Duplication of the original X chromosome has been suggested by Ohno, Becak and Becak (1964) as an explanation for the large X in some mammals. A hypothesis put forward by Matthey (1965a) is that a pair of homologous autosomes is translocated onto the primitive X and Y chromosomes resulting in large sex chromosomes.

In most mammals the XY association at meiosis is end to end (Nadler, 1969). However, if there was a translocation of homologous autosomes onto the sex chromosomes, a part side by side association would be expected to occur at meiosis, with possible formation of chiasmata between the homologous regions of the X and Y chromosomes. The XY association was therefore examined in meiosis of Mastomys with $2n = 36$ and $2n = 32$

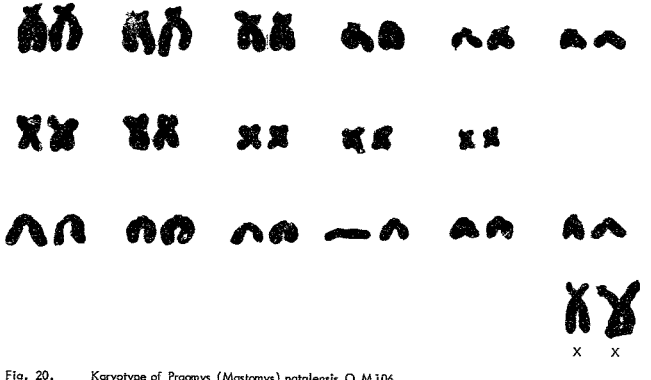


Fig. 20. Karyotype of Praomys (Mastomys) natalensis ♀ M106 from Lesotho.

chromosome complements. As colchicine injected mice were used for the meiotic studies in this investigation mostly first meiotic metaphase stages were to be seen on the slides.

3.2.1 Meiosis in the 2n = 36 chromosome group

The males had 18 bivalents in the first meiotic metaphase. Figures 22, 23, 24, 25 and 26 are photomicrographs of some of the meiotic metaphase plates obtained from the specimens. There were 17 autosomal bivalents and the XY bivalent in each plate. Identification of specific pairs among the autosomal bivalents was not possible, but the XY bivalent was one of the largest bivalents, usually identifiable by the difference in morphology and length of the two chromosomes involved. The XY association in some plates appeared to be part side by side with an interstitial chiasma (Figures 22, 23, 25 and 26). Other plates showed an end to end association which was probably the result of terminalization of the chiasma (Figure 24).

These observations are similar to those of Hamerton (1958) who found terminal association of the XY bivalent and also an XY bivalent with a subterminal chiasma in 2n = 36 Praomys (Mastomys) natalensis. Ford and Hamerton (1956b) noted the presence of a prominent unpaired interstitial loop in the sex bivalent of Mastomys at pachytene, with a terminal paired region at both ends. However no side by side association between both ends of the XY bivalent was seen in the present study. Huang and Strong (1962) suggested that the biggest bivalent contained the sex chromosomes in 2n = 36 chromosome Mastomys as it showed more pronounced contraction and separated earlier than the other bivalents at meiosis.



Fig. 22. Metaphase of spermatocyte meiosis in Praomys (Mastomys) natalensis M12 from the eastern Cape Province showing 18 bivalents. Arrow points to the XY bivalent.



Fig. 23. Metaphase of spermatocyte meiosis in Praomys (Mastomys) natalensis M17 from the southern Transvaal showing 18 bivalents. Arrow points to the XY bivalent.

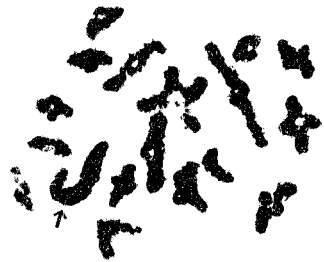


Fig. 24. Metaphase of spermatocyte meiosis in Praomys (Mastomys) natalensis M23 from the northern Cape Province showing 18 bivalents. Arrow points to the XY bivalent.

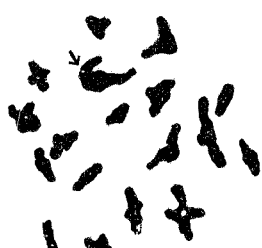


Fig. 25. Metaphase of spermatocyte meiosis in Praomys (Mastomys) natalensis M23 from the northern Cape Province showing 18 bivalents. Arrow points to the XY bivalent.



Fig. 26. Metaphase of spermatocyte meiosis in Praomys (Mastomys) natalensis M23 from the northern Cape Province showing 18 bivalents. Arrow points to the XY bivalent.

More pronounced contraction of the sex bivalent is evident in Figure 24. Early separation of the sex bivalent was not found, probably due to the inhibition of the spindle formation through the action of the colchicine.

The formation of interstitial chiasmata in the XY bivalents indicates the presence of fairly long homologous regions in the large X and Y chromosomes. This suggests to the author that there was an autosome-sex chromosome translocation in the evolution of Praomys (Mastomys) natalensis. This supports the hypothesis of Matthey (1965a) that large X and Y chromosomes have been formed by such translocation in some mammals.

3.2.2 Meiosis in the 2n = 32 chromosome group

There were 16 bivalents in the meiotic metaphase plates of the males and the XY was again the largest bivalent. Figures 27, 28, 29, 30 and 31 are photomicrographs of meiotic metaphase plates from two South West African specimens. The XY bivalents in some meiotic metaphase plates showed interstitial chiasmata (Figures 28 and 31). Other plates (Figures 27, 29 and 30) showed an end to end association between the X and Y chromosomes, which could be the result of terminalization of the chiasmata. It was not possible to associate any of the autosomal bivalents with particular chromosome pairs.

The same pattern of pairing between the X and Y chromosomes therefore exists in the two different chromosome groups of Praomys (Mastomys) natalensis. It would appear then that the chromosome rearrangements leading to the formation of the large X and Y chromosomes occurred in a common ancestor to both groups.

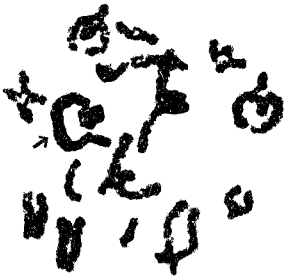


Fig. 27. Metaphase of spermatocyte meiosis in Praomys (Mastomys) natalensis M.20 from South West Africa showing 16 bivalents. Arrow points to the XY bivalent.



Fig. 28. Metaphase of spermatocyte meiosis in Praomys (Mastomys) natalensis M.20 from South West Africa showing 16 bivalents. Arrow points to the XY bivalent.

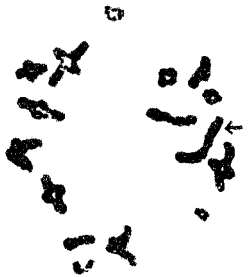


Fig. 29. Metaphase of spermatocyte meiosis in Praomys (Mastomys) natalensis M 20 from South West Africa showing 16 bivalents. Arrow points to the XY bivalent.



Fig 30. Metaphase of spermatocyte meiosis in Praomys (Mastomys) natalensis M 30 from South West Africa showing 16 bivalents. Arrow points to the XY bivalent.



Fig. 31. Metaphase of spermatocyte meiosis in Praomys (Mastomys) natalensis M30 from South West Africa showing 16 bivalents. Arrow points to the XY bivalent.

CHAPTER 4

DISCUSSION OF CHROMOSOME FINDINGS IN PRAOMYS (MASTOMYS) NATALENSIS IN SOUTHERN AFRICA

The results of the chromosome counts and karyotype analyses are summarized in Table 11. Also included in this table is the fundamental number ('nombre fondamental'=N.F. of Matthey, 1945) which refers to the number of major chromosome arms in a karyotype. It is a good indication of the link in a given taxonomic category and will be referred to later in the chapter.

Table 11. Summary of results of chromosome counts and morphological divisions of chromosomes from the Praomys (Mastomys) natalensis specimens obtained in southern Africa.

S = Submetacentric M = Metacentric A = Acrocentric

Area	Haploid Number	Diploid Number	Autosomes			Sex Chromosomes		N.F.	
			S	M	A	X	Y		
Eastern Cape	18	36	6	5	6	M	S	60	
Northern O.F.S.	18	36	6	5	6	M	S	60	
Southern Transvaal	18	36	6	5	6	M	S	60	
Lesotho	18	36	6	5	6	M	S	60	
Northern Cape	18	36	6	4	7	M	S	58	
								♀	♂
South West Africa	16	32	7	5	3	M	A	58	57
Northern Transvaal	16	32	7	5	3	M	A	58	57
Eastern Transvaal	16	32	7	5	3	M	A	58	57
Rhodesia	16	32	7	4	4	M	A	56	55

The results of the chromosome counts show that in southern Africa there are populations of Praomys (Mastomys) natalensis with two different chromosome numbers, a group to the south and towards the east with 36 chromosomes and a group to the north and spreading westwards with 32 chromosomes. The collections were too sparse to determine the precise distribution of these two groups, but the results show that the southern limit of the group with 32 chromosomes coincides with the known distribution limit of the species in South West Africa. In the Transvaal the results show that the 32 chromosome group occurs to the north and east, while in the south there is a 36 chromosome population. The specimens studied south of this collection point have 36 chromosomes. A red line drawn in the map in Figure 21 (Page 47) is an arbitrary line to show roughly where the division between the two groups lies.

4.1 Karyotypes of the 2n = 36 Chromosome Population

Mastomys with 36 chromosomes from the eastern Cape Province, northern Orange Free State, Lesotho and southern Transvaal all have karyotypes similar in appearance. This karyotype is the same as that described by Huang and [illegible]962) for a laboratory bred strain of Praomys (Mastomys) natalensis, which was bred from mice originating from Baragwanath, 16 kilometres south of Johannesburg in the southern Transvaal.

This more widespread karyotype among the 2n = 36 population is the following:-

6 pairs of submetacentric chromosomes

5 pairs of metacentric chromosomes

6 pairs of acrocentric chromosomes

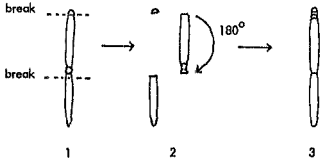
plus the sex chromosomes. Huang and Strong (1962) also encountered difficulty with the two smallest pairs in the submetacentric group, the short arms only being recognisable in clear plates or very early metaphase. Therefore in many cells it was difficult to distinguish those two pairs from acrocentric chromosomes of similar size (Huang and Strong, 1962).

The two large metacentric X chromosomes in the laboratory bred Mastomys female were found by Huang (1968) to be generally different in size and morphology when measured. He found one X to be slightly longer than the other; the larger X averaged 11.1μ, with an arm ratio of 1.5 while the other X averaged 9.2μ with an arm ratio of 1.2 in measurements of 12 cells. The X chromosome in the male cells resembled the larger of the two X's in the female.

As has already been discussed, the Y chromosome is a large submetacentric morphologically similar to the two largest pairs of submetacentric autosomes. This is similar to the findings of Huang (1968), though on actual measurement he found that the Y was intermediate in size between the 2nd and 3rd largest submetacentric autosome pairs.

Only the northern Cape Province Mastomys in the 2n = 36 chromosome group show a difference in their autosomal karyotype. These mice have only four pairs of metacentric chromosomes instead of the five pairs in those from the other areas, and an extra pair of chromosomes in the acrocentric group. A change in the position of the centromere in a metacentric chromosome is most likely due to a pericentric inversion. This is a turn about of a chromosome segment, which includes the centromere, through 180° so that the position is reversed. It involves two breaks in the chromosome, one on either side

of the centromere. In this particular instance the breakage and rearrangement could be diagrammatically represented as follows:-



This rearrangement brings about a reduction in the number of major chromosome arms by two in a homozygote for this change and would be reflected in Table 11 by the N.F. In the northern Cape Province specimens the N.F. is 58, while it is 60 for the other 2n = 36 Mastomys. This indicates that a pericentric inversion probably converted a pair of metacentric chromosomes into a pair of acrocentric chromosomes in the divergence of the northern Cape Province Mastomys population.

A pericentric terminal inversion involving only one break does not seem likely as most evidence favours the view that reunion without previous breakage does not occur (White, 1973).

Unless the two breaks are equidistant from the centromere on either side, pericentric inversions are readily detectable in mitotic metaphase chromosomes. Paracentric inversion, where the two breaks occur in the same chromosome arm, and an inversion occurs, is not observed in mitotic metaphase chromosomes.

4.2 Karyotypes of the 2n = 32 Chromosome Population

Mastomys with 32 chromosomes from South West Africa and the northern and eastern Transvaal all have karyotypes which appear to be similar with :-

7 pairs of submetacentric chromosomes

5 pairs of metacentric chromosomes

3 pairs of acrocentric chromosomes

plus the sex chromosomes. The X chromosomes in this group are also the largest chromosomes in the karyotype, and are metacentric. The Y chromosome is a large acrocentric, larger than the acrocentric autosomes. It is assumed by the author that this is the Y chromosome as it is a large acrocentric chromosome in the male karyotype with no homologous pair. With one sex chromosome being a metacentric and the other an acrocentric, there is a difference in fundamental number (N.F.) of one arm between the males and females in this 2n = 32 chromosome group (Table 11).

The Rhodesian Mastomys are the only group of 32 chromosome mice with an obvious difference in karyotype. These mice have only four pairs of metacentrics instead of the five pairs in the other 2n = 32 groups, and an extra pair of acrocentric chromosomes. This chromosome rearrangement is probably due to a pericentric inversion, the same as that described for the northern Cape Province Mastomys with 2n = 36 chromosomes. This similar pattern of chromosome rearrangement in two separated groups demonstrates White's (1973) principle of karyotypic orthoselection or homologous change in related groups or species. The pericentric inversion in the Rhodesian group is reflected in their lower fundamental number than the other 2n = 32

chromosome groups (Table 11).

4.3 Comparison between the Karyotypes of the Two Groups

A sample of the more generally widespread karyotypes in each of the two chromosome groups has been lined up in Figure 32 in order to demonstrate the difference in the karyotypes. The 2n = 36 chromosome karyotype is from a southern Transvaal male and the 2n = 32 chromosome karyotype is that of an eastern Transvaal male specimen. These two karyotypes were chosen for their similarity in size, rather than for clarity of the chromosomes, as the short arms of the smallest submetacentrics in the southern Transvaal male are not very clear.

Three pairs of acrocentric autosomes are missing from the 2n = 32 karyotype, and there is an extra pair of submetacentric autosomes. This extra pair of submetacentric autosomes suggests that there has been a *'Robertsonian' or centric type of fusion between two non-homologous* acrocentric chromosomes of different lengths to form this submetacentric chromosome. Controversy exists as to how this fusion occurs. It is usually regarded (White, 1973) as a special type of translocation involving a break near the centromere in each chromosome, one occurring on the long arm of one acrocentric and another on the submicroscopic short arm of the other acrocentric chromosome. Translocation then results in a bi-armed chromosome, and a centric and acentric fragment which are lost in subsequent cell divisions.

The diagram illustrating this kind of centric fusion appears on Page 63.

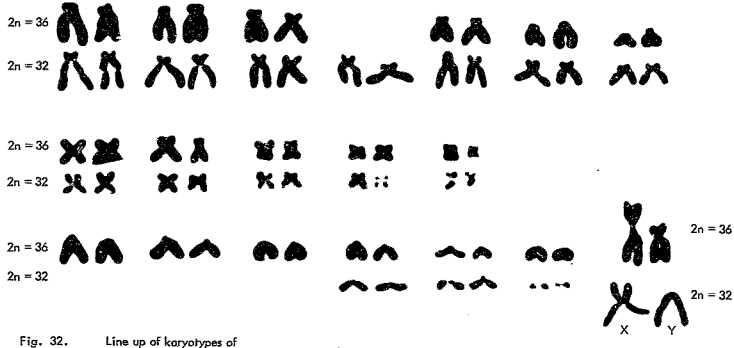
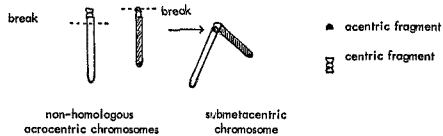


Fig. 32. Line up of karyotypes of
2n = 36 Praomys (Mastomys) natalensis ♂ from the southern Transvaal and
2n = 32 Praomys (Mastomys) natalensis ♂ from the eastern Transvaal
to demonstrate the differences between the karyotypes.

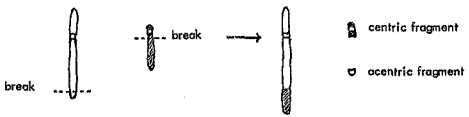


Matthey (1965a, 1973) is of the opinion that more frequently there is fusion of both centromeres of the two acrocentrics into a single one, which is closer to the original idea postulated by Robertson in 1916.

The possibility of fission of a submetacentric to form acrocentrics must also be considered, but since it requires the occurrence of centromere donors or a centromere splitting to form two functional centromeres it would appear to be a much rarer occurrence (Matthey, 1973).

In centric fusion (or fission) the number of major chromosome arms is not altered so the N.F. remains the same. However, the N.F. is different in the two groups, the females of the 2n = 32 chromosome group having two less major chromosome arms than the 2n = 36 group (Table 11). This is probably accounted for by a c[illegible] rearrangement involving the third extra acrocentric chromosome p[illegible] 2n = 36 group.

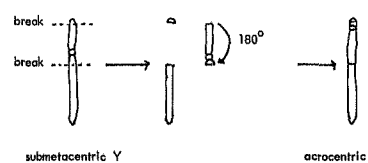
It is possible that the arm of an acrocentric chromosome was translocated onto the long arm of one of the smaller submetacentric chromosomes. Such a translocation would occur in the same manner as the reciprocal translocation type of centric fusion described above, but here the breaks in the chromosomes are not both near the centromeres. The following diagram illustrates this kind of reciprocal translocation.



A longer submetacentric is formed and the acentric and centric fragments are eliminated. In a homozygote for this type of translocation the N.F. would be reduced by two.

In the 2n = 32 karyotype (Figure 32) the largest pair of submetacentric chromosomes has an obvious secondary constriction approximately half way along the long arm. This shows up in most of the 2n = 32 karyotypes (Figures 11, 13, 15, 16 and 18), but is not present in the 2n = 36 karyotypes. Could this result be evidence of a weak link where a chromosome translocation has taken place?

The other difference between the karyotypes lies in the sex chromosomes. The Y chromosome is an acrocentric chromosome in the 2n = 32 karyotype and a submetacentric in the 2n = 36 karyotype. This is most probably due to a pericentric inversion converting a submetacentric chromosome to an acrocentric in the following manner:-



The chromosome rearrangements described are only one interpretation of what might have been the cause of the different chromosome number and N.F. between the two groups. This is the simplest explanation. During evolution of the two groups there may have been a more complicated pattern of change, but it would require more detailed analysis of the karyotypes and of individual chromosomes using banding techniques to establish the precise pattern of the chromosome rearrangements. The two groups may have come about as a result of chromosome rearrangements affecting the chromosomes of a common ancestor. As each group diverged its chromosomes probably changed from those of the common ancestor, which may have had 36 chromosomes or more.

4.4 External Morphology and Behaviour of the Two Chromosome Groups

The external morphology and characteristics of each of these groups of Praomys (Mastomys) natalensis are similar. Furthermore the different groups live in areas adjacent to each other. Hence one cannot infer that these are two separate species of mice. A difference in chromosome number and morphology between two individuals does not justify a conclusion that they be considered as two separate species (Hsu and Mead, 1969). Chromosome variation within a species is not uncommon (Mayr, 1963) as can be seen in the impala population of the Kruger National Park where there are three different chromosome numbers 58, 59 and 60 (Wallace and Fairall, 1967).

A behavioural difference among the different populations of mice became quite obvious after the author had handled them regularly.

The 2n = 32 chromosome group displayed a more frenzied activity when disturbed and generally seemed to be far quicker in their reactions than the 2n = 36 chromosome group. So striking was the difference that this level of reactivity was often a guide to their chromosome number even before it was determined.

Another difference, of which the author also became aware after constant observation of these mice, was a slightly more slender or more tapered snout in the 2n = 32 chromosome group than that in the 2n = 36 chromosome group.

This chromosome polymorphism in Praomys (Mastomys) natalensis is therefore indicative of either variation of chromosome number within a species, or of two separate species. To establish which of these two possibilities exists in the southern African Mastomys, it was decided to conduct breeding experiments between the two forms having 32 and 36 chromosomes.

CHAPTER 5

BREEDING EXPERIMENTS AND CONSEQUENT INVESTIGATIONS

5.1 Materials

The laboratory bred Baragwanath strain of Praomys (Mastomys) natalensis with 36 chromosomes was readily available for breeding purposes. The chromosomes of nine specimens from Rhodesia were examined and found to be 32 in number. It was then decided to keep the remaining three mice, two males and one female, from that area to use for hybridization experiments. Three crosses were therefore set up as follows:

Pair Numbers	Baragwanath Laboratory Strain (2n = 36)	Rhodesia (2n = 32)
1 X 2	♀	♂
3 X 4	♀	♂
5 X 6	♂	♀

Three mice received from South West Africa were all sacrificed for chromosome analysis. However, when a further batch of two mice (a male and a female) arrived from that area, it was decided to use them for breeding purposes first and to check their chromosome complements at a later stage. The male (M72) and female (M71) from South West Africa produced a litter within a month of their arrival, which made more mice with 32 chromosomes available for experimental breeding. The parents, M71 and M72, were subsequently found to have 32 chromosomes each, with identical karyotypes to those of the earlier batch from South West Africa, M20, M25 and M30 (see Figures 15 and 16). Five more males and females with different chromosome complements were therefore paired off as follows:

Pair Numbers			Baragwanath Laboratory Strain (2n = 36)	South West Africa (2n = 32)
I	X	II	♂	♀
III	X	IV	♂	♀
V	X	VI	♀	♂
VII	X	VIII	♂	♀
IX	X	X	♀	♂

5.2 Breeding Methods

The mice were paired off in standard metal mouse breeding cages, 30 cm X 15 cm X 13 cm in size. Their diet consisted of standard mouse cubes supplemented weekly with sunflower seeds, cabbage, carrots and potatoes. Water was supplied in drop bottles. The mice were kept in a temperature controlled room at about 21°C and were inspected daily for litters.

The aggressive and jumpy behaviour of the mice with 32 chromosomes made them very difficult to handle. Consequently these mice had to be transferred to clean cages in a large metal drum, from which escape was impossible.

The hyperactivity of the mice with 32 chromosomes was manifest also while they were undisturbed in their breeding cages. They would drag all their mouse cubes through their feeding baskets in a matter of hours, with the result that their cages filled up very rapidly and had to be changed often. The food baskets also became very light and the mice were then easily able to push the basket out of its socket and escape. To prevent this the cages had wide rubber bands put around them at the position of the food basket.

5.3 Results of Cross Breeding

The Baragwanath Laboratory Strain crossed with Rhodesian Mastomys produced litters within six weeks, as the mice used in these crosses were mature. When the South West African mice had matured at five to six months of age, the Baragwanath Laboratory Strain crossed with the South West African mice produced litters, with the exception of one pair, IX and X, of which the Baragwanath Laboratory Strain female died. On examination of the reproductive system of the dead female, no partly developed embryos or corpora lutea were seen. The breeding pairs were left together until they had had two or three litters so that there were many hybrids for investigation and further crossing. The litter sizes ranged from one to eleven and the interval between litters was 21 to 28 days, which is normal for Mastomys (Oliff, 1953). After the second or third litter the breeding pairs were separated. The male parents were moved to separate breeding cages and hybrid daughters were 'back-crossed' to their fathers, while hybrid sons were 'back-crossed' to their mothers. Some of the hybrids from each type of cross were removed for inspection and chromosome analysis, while the remaining F1 hybrids of each litter were left together as brother X sister matings in groups of twos and threes in separate breeding cages.

None of the father X daughter or mother X son 'back-crosses' produced any litters, nor did the brother X sister crosses, though they were together for up to eighteen months.

5.4 Phenotypic Appearance of the Hybrid

The hybrids were the brownish grey colour of the majority of Mastomys from southern Africa. The light pelage colouring, characteristic of the

South West African Mastomys was not seen in hybrids of crosses in which one parent was from that area. The mammae varied in number from 16 to 19, being the same as in the parental forms. The behaviour of the hybrids was clearly similar to that of the 32 chromosome Mastomys. They showed the same aggressiveness and frenzied activity of that group. They also resembled the 32 chromosome mice in having the same slender snouts. Though young adult hybrids were the same size as their parents, they gradually became much fatter as they grew older.

5.5 Chromosome Investigations in the Hybrids

The chromosomes of twelve hybrids were examined according to the methods described in Chapter 2. A male and a female hybrid from each original combination was examined as well as an extra hybrid from each combination. The sex of this extra hybrid was determined by what was available after the brother X sister matings and 'back-crosses' had been set up. The mice investigated were as follows:

Rhodesia ♂ X Baragwanath Laboratory Strain ♀ {1 ♂ 2 ♀♀
Rhodesia ♀ X Baragwanath Laboratory Strain ♂ {1 ♂ 2 ♀♀
South West Africa ♂ X Baragwanath Laboratory Strain ♀ {2 ♂♂ 1 ♀
South West Africa ♀ X Baragwanath Laboratory Strain ♂ {2 ♂♂ 1 ♀

Thirty cells in each hybrid were checked for their chromosome number and the metaphase plates scanned for the presence of the large acrocentric Y chromosome expected in the male hybrids with Rhodesian or South West African fathers. The chromosome counts are listed in Table 12.

Table 12. Chromosome counts of hybrids obtained from Baragwanath Laboratory Strain (Bara. L.S.) 2n = 36 crossed with Rhodesian (Rhod.) or South West African (S.W.A.) Mastomys 2n = 32

Mouse Number	Parents	Sex	Chromosome counts Number of cells with 33	34	Y Chromosome
M 35a	Rhod. ♂ X Bara.L.S. ♀	♂	-	30	acrocentric
M 34	" "	♀	1	29	-
M 35	" "	♀	-	30	-
M 101	Rhod. ♀ X Bara.L.S. ♂	♂	-	30	submetacentric
M 102	" "	♀	1	29	-
M 104	" "	♀	-	30	-
M 83	S.W.A. ♂ X Bara.L.S. ♀	♂	1	29	acrocentric
M 103	" "	♂	2	28	acrocentric
M 82	" "	♀	-	30	-
M 55	S.W.A. ♀ X Bara.L.S. ♂	♂	-	30	submetacentric
M 57	" "	♂	-	30	submetacentric
M 56	" "	♀	-	30	-
	Total	12	5	355	

All together the chromosomes of 360 cells were counted. All the hybrids had 34 chromosomes, with five cells out of the 360 showing a count of 33. Karyotypes were constructed from photomicrographs of the metaphase plates and karyotypes of a male and female representative of each group are shown in Figures 33 to 40.

No attempt was made to pair the chromosomes in these hybrid karyotypes as without critical 'banding' techniques one cannot be sure that any two of the chromosomes were members of the same homologous pair. Apart from the centric fusions and pericentric inversions which are postulated to have occurred in the

evolutionary divergence of the two parental groups (see Chapter 4), other not so obvious chromosome rearrangements such as paracentric inversions and reciprocal translocations have probably also occurred. The chromosomes were therefore separated into only the three morphological groups, and, as in the parental karyotypes, the separation of the smaller submetacentrics and the acrocentrics was arbitrary.

The karyotypes of hybrids from the Baragwanath Laboratory Strain crossed with Rhodesian Mastomys had

13 submetacentric chromosomes

9 metacentric chromosomes

10 acrocentric chromosomes

plus the two sex chromosomes (Figures 33, 34, 35 and 36). An acrocentric Y was present in the male hybrid, where the father was a Rhodesian Mastomys (Figure 33) and there was a large submetacentric Y chromosome where the father was a Baragwanath Laboratory Strain Mastomys (Figure 35). This was the chromosome complement to be expected from the haploid chromosome set contributed by each parent (see Table 13).

Table 13. Morphological division of hybrid chromosome complement with arrows indicating those chromosomes contributed by the haploid gametes of each parent.

	Baragwanath Lab. Strain (n = 18)		Hybrid chromosomes (2n = 34)		Rhodesia (n = 16)
Submetacentric	6	⟶	13	⟵	7
Metacentric	5	⟶	9	⟵	4
Acrocentric	6	⟶	10	⟵	4
	+ 2 sex chromosomes				

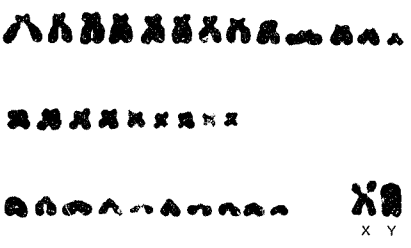


Fig. 33. Karyotype of a male hybrid from a Rhodesia ♂ (2n = 32) X Baragwanath Laboratory Strain ♀ (2n = 36).

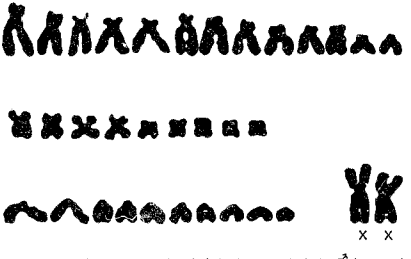


Fig. 34. Karyotype of a female hybrid from a Rhodesia ♂ (2n = 32) X Baragwanath Laboratory Strain ♀ (2n = 36).

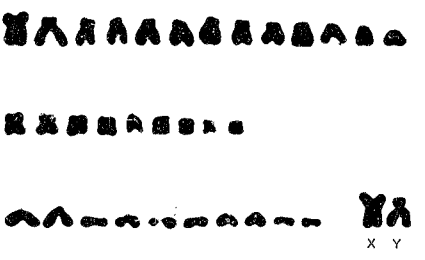


Fig. 35. Karyotype of a male hybrid from a Baragwanath Laboratory Strain ♂(2n = 36) X Rhodesia ♀ (2n = 32)

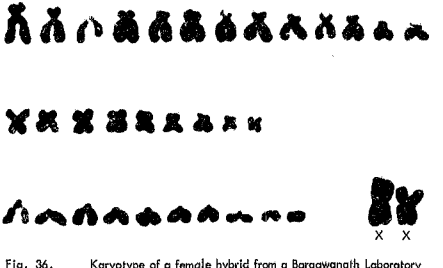


Fig. 36. Karyotype of a female hybrid from a Baragwanath Laboratory Strain ♂(2n = 36) X Rhodesia ♀ (2n = 32)

The hybrids from the South West African Mastomys crossed with the Baragwanath Laboratory Strain had

13 submetacentric chromosomes

10 metacentric chromosomes

9 acrocentric chromosomes

plus the two sex chromosomes (Figures 37, 38, 39 and 40). Where the Baragwanath Laboratory Strain provided the father, the Y chromosome of the male hybrids was a large submetacentric (Figure 37) and where the father was a South West African Mastomys the Y chromosome was a large acrocentric chromosome (Figure 39). This is again the chromosome complement one would expect in the hybrid when one considers the haploid chromosome set contributed by each parent (Table 14).

Table 14. Morphological division of hybrid chromosome complement with arrows indicating those chromosomes contributed by the haploid gametes of each parent.

	Baragwanath Lab. Strain (n = 18)		Hybrid chromosomes (2n = 34)		South West Africa (n = 16)
Submetacentric	6	→	13	←	7
Metacentric	5	→	10	←	5
Acrocentric	6	→	9	←	3
	+ 2 sex chromosomes				

5.6 Investigation into the Infertility of the Hybrids

As no litters were born of the back-crosses or brother X sister crosses, it appeared that the hybrids were sterile. The reproductive organs of the hybrids were therefore examined to determine whether there were any obvious signs of infertility. The methods of preparation of the specimens for examination are

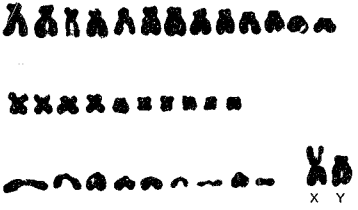


Fig. 37. Karyotype of a male hybrid from a Baragwanath Laboratory Strain ♂ (2n = 36) X South West Africa ♀ (2n = 32).

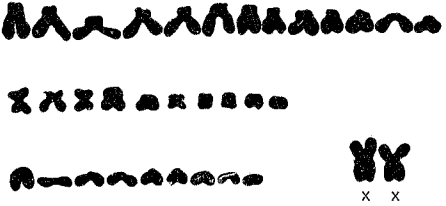


Fig. 38. Karyotype of a female hybrid from a Baragwanath Laboratory Strain ♂ (2n = 36) X South West Africa ♀ (2n = 32).

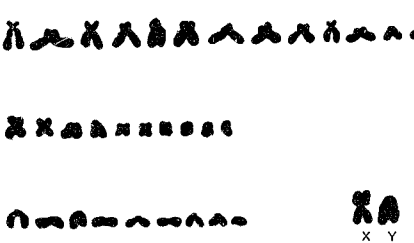


Fig. 39. Karyotype of a male hybrid from a South West Africa ♂ (2n = 32) X Baragwanath Laboratory Strain ♀ (2n = 36).

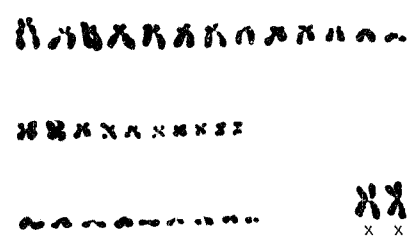


Fig. 40. Karyotype of a female hybrid from a South West Africa ♂ (2n = 32) X Baragwanath Laboratory Strain ♀ (2n = 36).

given in Chapter 2.

5.6.1 Male hybrids

The testes of the hybrids on first inspection were much smaller than those found in normal breeding mice of the same species. Large fat deposits were found in the abdominal cavity on dissection.

The weights of the testes in a series of hybrids were compared with those in a se ies of Baragwanath Laboratory Strain Mastomys from the breeding colony (Table 15).

Table 15. Comparison of paired testes weights of normal breeding Mastomys ♂'s and hybrid Mastomys ♂'s.

Normal breeding ♂ Baragwanath Lab. Strain		Hybrid ♂		
Mouse Number	Weight of paired testes	Number and origin of mouse		Weight of paired testes
M 90	1190mg	M73 Bara.L.S.	X S.W.A.	320mg
M 91	1490mg	M74 "	"	290mg
M 92	1400mg	M75 "	"	280mg
M 93	1230mg	M76 "	"	280mg
M 94	1460mg	M81 "	"	310mg
M 95	1420mg	M83 "	"	340mg
M 98	1185mg	M85 "	"	300mg
M 99	1170mg	M101 "	X Rhod.	280mg
M 100	1280mg	M103 "	X S.W.A.	340mg
Total (9)	11825mg			2740mg
Mean	1314mg			304mg

The testes of the hybrid mice were less than one quarter of the weight of the testes of normal breeding mice. In a study of wild living Praomys

(Mastomys) natalensis on the Transvaal highveld, the average paired testes weight of fecund mice was 890mg whilst that of unfecund mice was 350mg (Coetzee, 1967). The average paired testes weight in hybrid males in this investigation (304mg) compared favourably with the weight of 350mg in unfecund males. The average paired testes weight of laboratory breeding males was somewhat higher than in wild populations, but this could be expected considering the favourable food and temperature controlled conditions under which laboratory mice lived.

Microscopical examination of macerated cauda epididymidis tubules of all the hybrids showed no motile sperm. Cell suspensions of testicular material examined microscopically after treatment in isotonic sodium citrate and fixing showed no meiotic, but a little mitotic activity.

5.6.1.1 Histological sections

Haematoxylin and eosin stained sections of testicular material from normal and hybrid Mastomys were examined microscopically (Figure 41 to Figure 48). Figure 41 shows a section through the testis of a Baragwanath Laboratory Strain male. The tubules were full of spermatozoa and spermatocytes in various stages of meiosis. Figures 42 and 43 show sections through the testes of hybrid males M73 and M83. The tubules in the hybrids were obviously much smaller in diameter with more interstitial tissue between the tubules than in the normal male. Within the tubules there was a complete lack of spermatozoa, spermatids, secondary spermatocytes and post-pachytene stages of primary spermatocytes. There were some early stages of meiotic cell division up to the pachytene stage of the primary spermatocytes. Figure 44 shows the progression from early meiosis through to sperm formation in the



Fig. 41. Section through testis of a normal breeding ♂ Mastomys M95 of Baragwanath Laboratory Strain. Sectic shows seminiferous tubules with spermatozoa and spermatocytes in various stages of meiosis (X125).

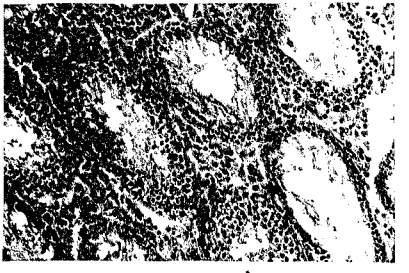


Fig. 42. Section through testis of hybrid ♂ M73 from cross between Baragwanath Laboratory Strain ♂ X South West Africa ♀ Mastomys. Section shows spermatocytes in early stages of meiosis, leptotene and zygotene. Note the smaller diameter of the tubules and increase of interstitial tissue when compared with the normal ♂ (X125).

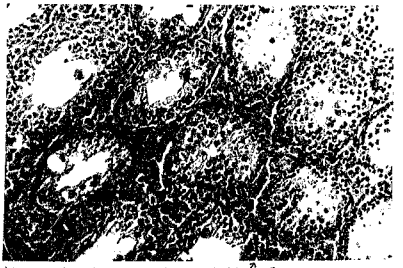


Fig. 43. Section through testis of hybrid ♂M 83 from cross between Baragwanath Laboratory Strain ♀ X South West Africa ♂ Mastomys. Section of testis shows spermatocytes in early stages of meiosis. A few cells here appear to be at early pachytene. The tubules are smaller with more interstitial tissue than the normal ♂ (X 125).

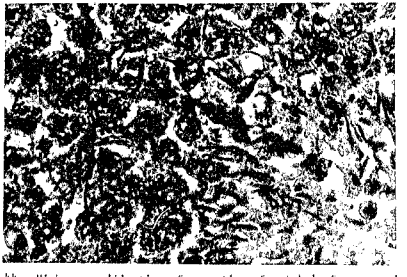


Fig. 44. Higher magnification of a portion of a tubule from normal ♂ in Fig. 41. Spermatocytes in various stages of meiosis can be seen and spermatozoa towards the centre of the tubule (X 500).

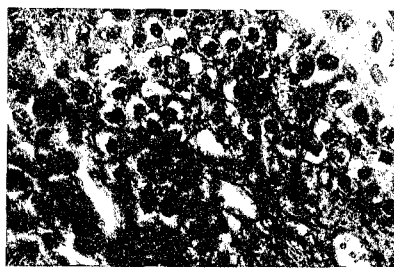


Fig. 45. Higher magnification of a portion of a tubule in the testis of hybrid ♂ M73 in Fig. 42. This shows more clearly the spermatocytes in the leptotene-zygotene stages (X500).

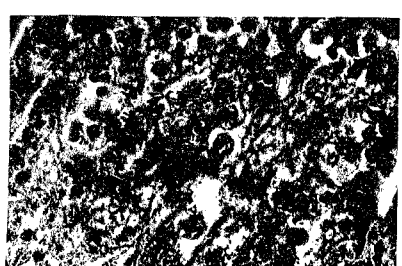


Fig. 46. Higher magnification of a portion of a tubule in the testis of hybrid ♂ M83 in Fig. 43. This shows stages in meiosis up to early pachytene in the spermatocytes (X500).



Fig. 47. Section through the epididymis of normal ♂ Mastomys M95 showing tubules filled with spermatozoa (X125).

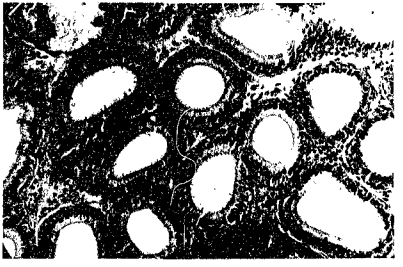


Fig. 48. Section through the epididymis of hybrid ♂ M83 showing small empty tubules surrounded by interstitial connective tissue (X125).

tubule of a normal Baragwanath Laboratory Strain male. Figures 45 and 46 show the situation in hybrid tubules. In M73 (Figure 45) the chromosomes appeared to be no further than zygotene, while in M83 (Figure 46) there were well established early pachytene stages before the onset of the growth phase.

Figure 47 shows a cross section of the epididymis of a normal breeding male Baragwanath Laboratory Strain Mastomys with the tubules full of spermatozoa. Figure 48 is a section of the epididymis of a hybrid male (M83) in which the empty tubules were of a much smaller diameter than those of the normal male and there was more interstitial tissue.

5.6.2 Female hybrids

When the abdominal cavity of the females was dissected open a mass of fatty tissue was seen. The ovaries and uteri of the hybrids appeared similar to those of normal breeding adult female Mastomys of the Baragwanath Laboratory Strain. However, as these organs are usually fairly small, it was decided to weigh them in order to detect any differences. The weights of ovaries and uteri in a series of seven hybrids were compared with those in a series of five normal breeding Baragwanath Laboratory Strain adults (see Table 16). The table indicates that there was a small difference in the ovarian weights between the two groups, the hybrid ovaries being slightly larger. There was however a more marked difference in the uterine weights. The uterus in the hybrids was almost double that of the normal breeding female, 208 gm in the hybrid as compared with 109 gm in the normal Mastomys.

Table 16. Comparison of the weights of ovaries and uteri in normal breeding Mastomys ♀'s and hybrid Mastomys ♀'s.

Normal Breeding ♀ Baragwanath Laboratory Strain			Hybrid ♀		
Mouse Number	Weight of Uteri	Ovaries	Number and origin of mouse	Weight of Uteri	Ovaries
M87	150mg	50mg	M77 Bara.L.S. X S.W.A.	180mg	70mg
M88	100mg	50mg	M78 " " "	190mg	70mg
M89	100mg	50mg	M79 " " "	200mg	40mg
M96	80mg	60mg	M80 " " "	270mg	80mg
M97	115mg	65mg	M82 " " "	220mg	80mg
			M84 " " "	260mg	70mg
			M86 " " "	135mg	50mg
Total (5)	545mg	275mg	Total (7)	1455mg	460mg
Mean	109mg	55mg		208mg	66mg

5.6.2.1 Histological sections

Haematoxylin and eosin stained sections of hybrid ovaries showed a rather disturbed picture in comparison with that of a normal ovary. Figure 49 shows a section through the ovary of a normal mature adult Baragwanath Laboratory Strain Mastomys female, showing immature follicles with oocytes and corpora lutea probably degenerating. Figures 50, 51 and 52 show sections through three hybrid ovaries. In the hybrid ovaries there were what appeared to be groups of atypical follicle cells and also some corpora lutea, but there were no immature follicles showing clearly the oocyte surrounded by follicle cells to be seen in any of the ovaries examined.

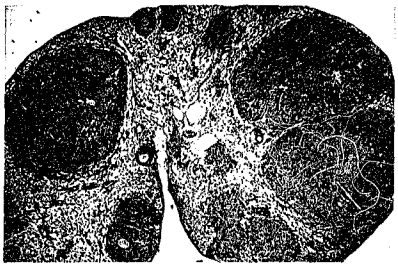


Fig. 49. Section through ovary of normal mature Baragwanath Laboratory Strain Mastomys ♀ M96 (X 31.25).

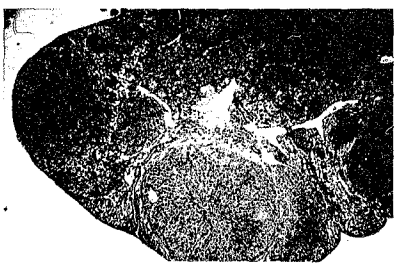


Fig. 50. Section through ovary of hybrid ♀ M86 from cross between Baragwanath Laboratory Strain ♂ x South West Africa ♀ Mastomys (X 31.25).



Fig. 51. Section through ovary of hybrid ♀ M79 from cross between Baragwanath Laboratory Strain ♂ X South West Africa ♀ Mastomys (X 31.25).



Fig. 52. Section through ovary of hybrid ♀ M84 from cross between Baragwanath Laboratory Strain ♂ X South West Africa ♀ Mastomys (X 31.25).

5.7 Discussion

Hybrids are produced from Mastomys parents with 36 chromosome and 32 chromosome complements. There is no noticeable reduction in litter size obtained from crossing animals of these two chromosome groups, nor is there any altered sex ratio in the litters.

The hybrids are similar in appearance to the parents, but though on maturing they are initially the same size as the parents, as they get older they tend to get much fatter. Large deposits of fatty tissue are found in the abdomen. In one respect, however, the behavioural pattern of the hybrids is that of the 32 chromosome group as they are equally aggressive and as jumpy as their 32 chromosome parent. Their snouts are also slightly more tapered than in the 36 chromosome Mastomys, resembling more the 32 chromosome type. The hybrids are infertile when 'back-crossed' to the parents or in sibling crosses.

The chromosome complement of $2n = 34$ in the hybrid is made up of a haploid set of chromosomes contributed by each parent. The reproductive systems of the hybrids are affected by this combination. The normal development of the gonads is disrupted and also the meiotic processes which occur within them. The gonads in the male are small and underdeveloped, while those of the female are increased in size.

The testes are less than a quarter of the size of those in normal breeding males and contain tubules of a much smaller diameter, with an increase in the amount of interstitial tissue between them. The uterus of the hybrid female is double the weight of that in a normal breeding female while the ovary is only slightly bigger.

However, though the testes are small and underdeveloped, the beginnings of spermatogenesis are seen in the seminiferous tubules. It is the breakdown in the process of meiosis which apparently results in the non-formation of secondary spermatocytes, spermatids and spermatozoa and hence in the hybrid infertility. The breakdown seems to occur in the pairing of the chromosomes at pachytene in the hybrids since no post-pachytene cells were seen.

The pattern of development in the ovary of the female hybrid seems to be quite irregular. It is probable that oogonia are formed, but it cannot be established from sections of the ovaries whether oocytes are present. The surface epithelium does give off sterile follicle cells, which appear to respond to pituitary gonadotrophins and corpora lutea are formed. As the nucleus of the oocyte enters into the prophase of meiotic divisions simultaneously with the growth of the oocyte (Balinsky, 1960), it is possible that this is why no developing oocytes are seen. Meiosis probably comes to the same standstill in the oocytes as in the primary spermatocytes; and, though subsequent stages of meiosis are postponed until the end of the period of growth, the inability to complete the first stage may prevent the growth of the oocyte.

The great increase in the size of the uterus of the hybrid, when compared with the normal is most likely due to a hormonal imbalance. The fact that follicular cells are formed in the ovary but no ova are ever released, suggests that there may be a build up of oestrogen by the follicular cells. As the corpus luteum in rodents does not become functional and immediately begin to secrete progesterone unless pregnancy ensues (Bullough, 1951)

it is possible that the progesterone level in the infertile hybrids is low. Oestrogen has been found to cause increased vascularization and greater mitotic activity of the uterus in castrated or infantile females, which results in a great increase in the weight of the uterus (Nalbandov, 1958). Oestrogen therapy in rats and mice also leads to accumulation of water in the uterine lumen (Nalbandov, 1958) which would also significantly affect the uterine weight.

5.7.1 Mechanism of breakdown in meiosis

Meiosis is seen to proceed in the primary spermatocyte as far as the pachytene stage. This means that the homologous chromosomes have already become closely approximated side by side, as a result of the process of synapsis, during the zygotene stage. As meiosis proceeds no further than pachytene it would seem that incomplete pairing of chromosomes at this stage prevents the process of meiosis from advancing.

The possibility that centric fusion has occurred between two acrocentric chromosomes in the 2n = 32 chromosome Mastomys (as proposed in Chapter 4) means that a trivalent in the hybrid could be expected between the two acrocentrics, and the fusion submetacentric. White (1973) has suggested, though, that a trivalent in this instance may be realized in only a minority of cells, the remainder having a bivalent and a univalent. A trivalent (or bivalent and univalent) would also be expected to occur in a hybrid, where a translocation has taken place in one of the parent species, as it is suspected in the 2n = 32 chromosome Mastomys (see Chapter 4).

Where there is a Rhodesian 2n = 32 chromosome parent in some of the crosses there is also a pericentric inversion in one of the haploid complements

making up the hybrid karyotype. In an homologous pair of chromosomes in an individual heterozygous for a pericentric inversion, complete synapsis can lead to a reversed loop at pachytene. However if an inversion is sufficiently short the chromosomes might be too rigid to form such a reversed loop and synapsis between the inverted region would not occur (White, 1973).

Even if it is theoretically possible for meiosis to proceed with the three described chromosome rearrangements, it is not known how many undetected complex structural chromosome rearrangements have occurred in each of the parental forms since their divergence. Hence it is not possible to estimate how many complete synapses of homologues to form bivalents have taken place in the hybrid, nor is it possible to distinguish any of this detail from the dividing spermatocytes.

There is no direct evidence that the same situation, of arrest at prophase I of meiosis, exists in the female oogonia. It is however to be expected that there would be the same obstructions to pairing of homologous chromosomes as exists in the hybrid spermatocytes, if the female germ cell development proceeds that far.

5.7.2 Male hybrids in some other mammals

A similar pattern of male hybrid infertility has been found fairly frequently in males from crosses between closely related species in a number of different orders of mammals. Perhaps the most frequently studied are the Equid hybrids. Mules, or hybrids from horse (Equus caballus 2n = 64) X donkey (E. asinus 2n = 62) parents, and zebroids from Grant's zebra (E. burchelli 2n = 44) X donkey parents and from Grevy's zebra (E. grevyi 2n = 46) X horse parents showed a similar histological

appearance of the testes to that described earlier in this chapter for Mastomys hybrids; namely seminiferous tubules with smaller diameters than in the parent species, incomplete spermatogenesis with failure of meiosis at the pachytene to diplotene stages (Benirschke, 1967, King, 1967).

Within the order of Rodentia other sterile hybrid males with similar histological patterns have been produced. Hybrids derived from interspecific crosses in the genus Meriones were infertile. Disturbed meiosis was found in F1 males of M. crassus (2n = 60) ♀ X M. tristrami (2n = 72) ♂ by Zahavi and Wahrman, 1957. The male hybrid from M. libycus (2n = 44) ♀ X M. tristrami (2n = 72) ♂ showed no spermatogenesis or spermatozoa (Matthey, 1957). Lay and Nadler (1969) crossed two species of Meriones (M. shawi and M. libycus which are partially sympatric), with the same chromosome number (2n = 44), but with different karyotypes and differences in gross morphological features. The males were infertile with absent or diminished spermatogenesis and arrest of meiosis in prophase 1, though the seminiferous tubules did not have a smaller diameter than that of normal parents. One specimen displayed spermatogenesis but to a lesser degree than the male parent. In three other hybrids a few seminiferous tubules contained spermatids and infrequent, round-headed, atypical spermatozoa, while a few others had secondary spermatocytes or spermatids but no spermatozoa.

5.7.3 Female hybrids in some other mammals

Less information is available on the female hybrids. They are more frequently fertile than male hybrids. Often female hybrids are the only hybrids produced or there is a reduction in the production of male hybrids in mammals, which is attributed to their being the heterogametic sex (Craft, 1938).

Benirschke and Sullivan (1966) found that corpora lutea were produced relatively infrequently in the ovaries of female mules, and primordial ova were extremely uncommon in 94 ovaries examined. This led to the suggestion that the female germ cells may have incited the development of granulosa cells, which later responded to pituitary gonadotrophins normally, while the germ cell itself died in meiosis (Benirschke and Sullivan, 1966, Benirschke, 1967). A similar pattern is described in this investigation of the female Mastomys hybrids.

The F1 females of a Meriones shawi ($2n = 44$) X M. libycus ($2n = 44$) cross were fertile (Lay and Nadler, 1969). The F1 females back-crossed with M. shawi males were fertile and produced normal sized litters with normal sex ratios; while the F1 females back-crossed with M. libycus males exhibited reduced fertility, small litters and an absence of male offspring.

5.7.4 Systematic implications of results

The infertile hybrids investigated have for the most part been interspecific hybrids between species with different karyotypes and differences in gross morphological features. The situation in Mastomys described here is different in that one is dealing with mice with similar morphological features and which have therefore been considered as one species ranging throughout southern Africa. Only recently Setzer, (1975 and personal communication) has found two separate groups within a Praomys (Mastomys) natalensis population from the Mkuze area of northern Natal, based on measurements of the rostral portion of the skulls. However the evidence presented here does show that two very distinct groups are present in southern Africa. These groups are genetically isolated from each other in that

only infertile hybrids are produced when members of the two groups are crossed experimentally, so that in effect a post-mating, reproductive isolating mechanism operates.

The ability to form distinct chromosome races is far more likely to occur in species which have a fairly low vagility. The maximum recorded home range of Praomys (Mastomys) natalensis is 4800m^2 with mean home ranges of 2666m^2 for males and 1833m^2 for females (De Wit, 1972). With its semi-domestic nature, Mastomys is able to occupy a fairly wide variety of habitats and are ecologically tolerant enough to be able to shift to a variety of niches and colonize new geographical regions. It is to be expected therefore that there are distinct chromosomal races amongst Mastomys.

Groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups are recognized as species according to Mayr (1963). In many well investigated groups of mammals and insects, the evidence points rather clearly to cytological races as the main source of incipient species (White, 1973), but White points out that cytotaxonomic differences may have come about after speciation, through chromosome rearrangements arising in young or ancestral specimens. In Mastomys because it is the chromosome rearrangements which prevent meiosis proceeding through to the formation of sperm and possibly of ova in a hybrid, it is more likely to be an example of the former situation in which a cytological race was the potential source of an incipient species. As the two different groups of Mastomys have not developed clear morphologically distinguishable characteristics, they should perhaps be considered as sibling species. By definition these are morphologically similar or identical populations that are

reproductively isolated (Mayr, 1963). There is, of course, already a difference in the behaviour of the two races and, in a natural environment, ethological isolation might prevent even the production of natural hybrids, whether the two groups were allopatric or sympatric.

Natural hybrids have been collected at points of contact between burrowing rodents of the complex Spalax ehrenbergi. Hybrids were found between the 52 and 54 chromosome group and between the 58 and 60 chromosome group (Wahrman, Goitein and Nevo, 1969a, 1969b). Nevo (1969) suggested there was some degree of ethological isolation between the two groups, in that there is more pronouncedly aggressive behaviour and a lower frequency of copulation between the heterogametic pairs, when compared with the homogametic pairs.

5.8 Conclusion

The cytogenetic evidence presented here suggests that chromosomal rearrangements have played a direct causal role in the evolution of Praomys (Mastomys) natalensis in southern Africa. As the formation of new species is a gradual process, it is difficult to decide when to designate a particular form as a race, a sibling species or a full species. What is more significant than the terminology thereof is the fact that extensive karyotypic divergence exists in what was thought by classical taxonomical methods to be a single uniform monomorphic species. This is in accord with Tobias' concept of multiformity in chromosome evolution, or evolutionary divergence closely paralleled by change in chromosome numbers. Multiformity is one trend found in mammalian evolution, while the other is the concept of uniformity, or morphological divergence with no change in chromosome numbers (Tobias, 1953).

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

The classification of Praomys in southern Africa is relatively simple. Only three species of the genus are recognised, P. verreauxi, P. natalensis and P. shortridgei (Meester, 1964, Misonne, 1968). P. verreauxi is placed in the subgenus P. (Myomyscus) verreauxi as it has different morphological characteristics from the other two species, the most obvious being the longer tail and narrower teeth. The other two species are placed in the subgenus Mastomys, which has a tail usually shorter than the head and body and rather broad molars.

Of the two P. (Mastomys) species only P. (Mastomys) natalensis has a very wide distribution that includes most of southern Africa except the western Cape Province. Meester (1964) gives the range of natalensis as eastern and northern Cape Province, Natal, Orange Free State, Lesotho, Transvaal, Swaziland, Mozambique, southern Rhodesia, northern Botswana, Caprivi Strip and northern South West Africa (see Figure 1 at end of Chapter 1). P. (Mastomys) shortridgei is found only in the Okavango region of northern South West Africa where it occurs together with natalensis. The two species are very similar but shortridgei has 10 mammae where natalensis has 12 or more, and the colour of shortridgei is darker than natalensis (Meester, 1964, Misonne, 1968). Several subspecies of P. (Mastomys) natalensis have been described but are doubtfully valid (Meester, 1964) as they are based mainly on colour variation of the pelage.

P. (Mastomys) natalensis in southern Africa has therefore been considered a single species on morphological grounds. All chromosome counts done previously on these Mastomys have shown a diploid number of 36 chromosomes (Matthey, 1954, 1966a, Ford and Hamerton, 1956b, Huang and Strong, 1962). Those investigated by Matthey and Huang and Strong were Baragwanath Laboratory Strain Mastomys but the origin of that investigated by Ford and Hamerton is unknown to the author. P. (Mastomys) natalensis with a diploid number of 32 chromosomes has been reported from southern Africa for the first time in this investigation, though they have been found in Central and West Africa.

6.1 *Karyotypes of 2n = 32 Chromosome Group in Sub-Saharan Africa*

A 2n = 32 chromosome form of a Praomys (Mastomys) sp. has been known as far south as the Congo-Brazzaville (Matthey, 1966a) and has also been found in areas investigated to the north of this region. These areas are the Central African Republic (Matthey, 1955, 1966a) the Ivory Coast (Matthey, 1966a, Bellier, 1975) and Chad (Matthey, 1966b). In the present study diploid counts of 32 chromosomes have been found in Mastomys in areas much further south in the African continent, namely South West Africa, Rhodesia and the northern and eastern Transvaal.

The autosomal karyotypes of those Mastomys from the Congo-Brazzaville, Central African Republic and the Ivory Coast are similar to those described here for specimens from South West Africa and the northern and eastern Transvaal. *There are only three pairs of acrocentric autosomes in each karyotype and* twelve pairs of meta and submetacentric autosomes in each. Though Matthey

(1966a) does not distinguish between meta and submetacentric autosomes in his karyotypes of Central and West African mice, from a personal study of the karyotypes presented it appears to the author that five pairs are metacentric as is found in the South West African and northern and eastern Transvaal Mastomys. The only obvious difference between the two groups is in the Y chromosome. In the specimens from Central and West Africa the Y is a large submetacentric, whereas the southern group have a large acrocentric Y. This is most likely due to a pericentric inversion converting a submetacentric Y to an acrocentric, as described in Chapter 4. The X chromosome in all the 2n = 32 groups is a very large metacentric chromosome.

The 32 chromosome group in Rhodesia, however, has a slightly different karyotype, with four pairs of acrocentric autosomes instead of three as in the other southern groups. This extra pair of acrocentric autosomes was possibly due to a pericentric inversion affecting a pair of metacentric autosomes, as there is one less pair in this group.

This type of pericentric inversion was very well demonstrated in a group of Mastomys from Fort Lamy, Chad, studied by Matthey (1966b). There the change was in a very active state as Matthey found two heterozygotes for the pericentric inversion which had 7 acrocentric autosomes (3 pairs + 1 single); four specimens with 4 pairs or 8 acrocentric autosomes (as found in the Rhodesian specimens) and one mouse with only 3 pairs or 6 acrocentric autosomes (as found in all other Mastomys with 32 chromosomes). The Chad Mastomys had a large submetacentric Y similar to the other northern groups.

The above information is tabulated in Table 17 for easy reference and comparison. Also listed in this table is the N.F. (fundamental number, which

Table 17. A comparison of chromosome types and N.F. in Praomys (Mastomys) natalensis with 32 chromosomes from different areas in sub-Saharan Africa.

Diploid number	Origin of mice	Submetacentric and metacentric autosomes	Acrocentric autosomes	X chromosome	Y chromosome	N.F.	Reference
32	Central African Republic	12 pairs	3 pairs	metacentric	submetacentric	58	Matthey, 1966a
32	Congo-Brazzaville	12 pairs	3 pairs	" "	" "	58	Matthey, 1966a
32	Ivory Coast	12 pairs	3 pairs	" "	" "	58	Matthey, 1966a
32	Chad (Fort Lamy)	11 pairs + 1	3 pairs + 1	" "	" "	57	Matthey, 1966b
		11 pairs	4 pairs	" "	" "	56	Matthey, 1966b
		12 pairs	3 pairs	" "	" "	58	Matthey, 1966b
32	Rhodesia	11 pairs	4 pairs	" "	acrocentric	(55 ♂ (56 ♀	Present investigation
32	South West Africa	12 pairs	3 pairs	" "	" "	(57 ♂ (58 ♀	" "
32	Northern Transvaal	12 pairs	3 pairs	" "	" "	(57 ♂ (58 ♀	" "
32	Eastern Transvaal	12 pairs	3 pairs	" "	" "	(57 ♂ (58 ♀	" "

is explained in Chapter 4) which ranges between 55 and 58. The N.F. in the most widespread karyotype is 58; in the heterozygote for one pericentric inversion it is one less, 57, while in the homozygote for one pericentric inversion it is 56. In the southern group where a pericentric inversion has converted the Y to an acrocentric chromosome, the N.F. in the male is one less than that of the female.

6.2 Karyotypes of 2n = 36 Chromosome Group in South Africa

The karyotype patterns found amongst the 2n = 36 chromosome Mastomys are shown in Table 18. There is some difference between the karyotype described by Huang and Strong (1962) and that described by Matthey (1966a) for the Baragwanath Laboratory Strain mice. They show different numbers of autosomes in the combined submetacentric and metacentric group and in the acrocentric group. This could be due to the different authors' interpretation of acrocentric or 'telocentric' chromosomes, variations in methods of chromosome preparation and differences in degree of contraction of the metaphase chromosomes. In fact the two different karyotypes found by Matthey were from cells in the same animal in each of two mice. It must also be remembered, however, that specimens studied by the two authors are different generations (far apart) of an inbred Mastomys colony, where once a chromosome rearrangement has occurred it would not be long before it could be established in the homozygous state.

In the present investigation there is one fairly widespread karyotype found with eleven pairs of submetacentric and metacentric autosomes and six pairs of acrocentric autosomes, the same as that described by Huang and Strong (1962). In this karyotype there are two small submetacentrics, in

Table 18. A comparison of chromosome types and N.F. in Praomys (Mastomys) natalensis with 36 chromosomes from different areas in southern Africa.

Diploid number	Origin of mice	Submetacentric and metacentric autosomes	Acrocentric autosomes	X chromosome	Y chromosome	N.F.	Reference
36	Baragwanath Laboratory Strain	11 pairs	6 pairs	metacentric	submetacentric	60	Huang and Strong (1962)
36	Baragwanath Laboratory Strain	9 pairs	8 pairs	" "	" "	56	Matthey, 1966a
36	Baragwanath Laboratory Strain	10 pairs	7 pairs	" "	" "	58	Matthey, 1966a
36	Southern Transvaal	11 pairs	6 pairs	" "	" "	60	Present investigation
36	Eastern Cape Province	11 pairs	6 pairs	" "	" "	60	" "
36	Northern Orange Free State	11 pairs	6 pairs	" "	" "	60	" "
36	Lesotho	11 pairs	6 pairs	" "	" "	60	" "
36	Northern Cape Province	10 pairs	7 pairs	" "	" "	58	" "

which the short arms are very small but recognizable in clear plates. The N.F. of these Mastomys is 60, while that of the northern Cape Province specimens is 58. This is explained by a pericentric inversion converting a pair of metacentric autosomes to a pair of acrocentric autosomes, as described in Chapter 4. The X chromosomes in all the specimens described are the largest chromosomes in the karyotype and are metacentric. The Y chromosome is a large submetacentric in all the specimens.

6.3 Karyotypes of 2n = 38 Chromosome Group in West Africa

The other Praomys (Mastomys) which has been investigated cytogenetically is what is now considered to be a separate species from ·.tatensis, Praomys (Mastomys) erythroleucus on both morphological (Petter, 1957) and karyological grounds (Matthey, 1958, 1965b, 1966a, Bellier, 1975). This Mastomys from the Ivory Coast has 38 chromosomes and is partially sympatric with the 2n = 32 chromosome group. The number of ch each group and the N.F. is given in Table 19. Most of the speci three males and a female had seven pairs of submetacentric and metacentric autosomes and eleven pairs of acrocentric autosomes (Matthey, 1966a). One female had two cell lines, one with ten pairs of acrocentric autosomes and the other with ten pairs plus one extra acrocentric autosome. Matthey suggested this might be due to a pericentric inversion in one lymphoblastic cell line. This possible pericentric inversion accounts for the variation in N.F. between 54 and 56. The X and Y chromosomes are similar to those found in the 2n = 36 chromosome group, i.e. a very large metacentric X and a large submetacentric Y chromosome.

Table 19. A comparison of chromosome types and N.F. in Praomys (Mastomys) erythroleucus with 38 chromosomes from West Africa.

Diploid number	Origin of mice	Submetacentric and metacentric autosomes	Acrocentric autosomes	X chromosome	Y chromosome	N.F.	Reference
38	Ivory Coast	7 pairs	11 pairs	metacentric	submetacentric	54	Matthey, 1966a
		8 pairs	10 pairs	"	" "	56	Matthey, 1966a
		7 pairs + 1	10 pairs + 1	"	" "	55	Matthey, 1966a

6.4 Relationship between the Different Chromosome Groups

Hybrids were produced from crosses between the 2n = 32 and the 2n = 36 chromosome groups, as described in Chapter 5. These hybrids were sterile and an effective post-mating isolating mechanism is therefore operative between these two morphologically similar groups. For this reason the author considers that the 2n = 32 and the 2n = 36 chromosome groups should be referred to as sibling species. Praomys (Mastomys) erythroleucus with 2n = 38 chromosomes is already considered a separate species from the other two groups (see 6.3). This has been substantiated by breeding experiments; crosses between the 2n = 32 and 2n = 38 chromosome groups, and between the 2n = 36 and 2n = 38 chromosome groups have failed to produce any offspring (Dickson, 1967).

The number of major chromosome arms (N.F.) is a good indication of the link in a given taxonomic category. The N.F. in the three different chromosome groups ranges from 54 to 60. Changes in chromosome number without a change in N.F. are indicative of Robertsonian fusions/fissions having occurred. Although centric fusions have played an important part in the evolution of Mastomys (Matthey, 1966a) the difference in N.F. is indicative of the occurrence of other structural chromosome rearrangements. Both pericentric inversions and translocations cause a change in N.F. and both of these rearrangements have been postulated to have occurred.

The fact that pericentric inversions have occurred independently in each of the three chromosome groups discussed is of interest. In each case the pericentric inversion involves conversion of a metacentric chromosome to an acrocentric chromosome, or vice versa, which demonstrates White's

(1973) principle of homologous change or Tobias' (1956) principle of parallelism, whereby species in related groups have undergone the same type of change.

The X and Y chromosomes in each of the groups are unusually large. The X chromosomes in the females and the single X in the male are always the largest metacentric chromosomes in the karyotype. The Y in most of the groups is a large submetacentric chromosome. Only in the southern 2n = 32 chromosome group is the Y a large acrocentric, probably due to pericentric inversion.

Meiotic studies in Chapter 3 on 2n = 32 and 2n = 36 groups in southern Africa showed part side by side association of some XY bivalents. This indicated to the author that a pair of homologous autosomes had been translocated onto the original X and Y chromosomes, thus accounting for their large size. This mechanism of autosome-sex chromosome translocation was postulated by Matthey (1965a) in his studies on Leggada. As similar large sex chromosomes are found in all the 2n = 32, 2n = 36 and 2n = 38 chromosome Mastomys studied, such a translocation could have occurred in a common ancestor to all three groups. However, part side by side association of the XY bivalent was not shown by Matthey in his studies of meiosis in these groups of Mastomys (1954, 1955, 1958, 1966a), though it was demonstrated by Hamerton (1958) in 2n = 36 Mastomys.

The possible phylogenetic relationship and chromosome rearrangements in the 2n = 32 and 2n = 36 groups are represented diagrammatically in Figure 53. As a personal study of the 2n = 38 chromosome group has not been made by the author, this group is not included in the diagram.

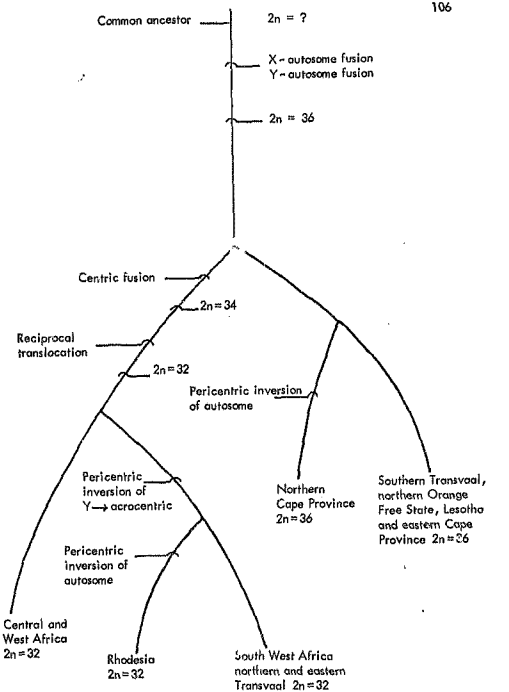


Fig. 53. Diagram of possible phylogenetic relationship and chromosome rearrangements in 2n = 32 and 2n = 36 Mastomys.

6.5 Geographic Distribution

A provisional geographic distribution map of the three chromosome groups was given in Matthey in 1966a. This map is reproduced here in Figure 54. The southern portion of this map has changed considerably with the information gathered in this investigation. Figure 55 is a similar outline map of Africa showing the new reduced extent of the 36 chromosome range and a new known southern limit of the 2n = 32 chromosome group.

It is possible that the 2n = 32 chromosome group extends, or once extended from the Congo-Brazzaville area through the chromosomally uninvestigated areas of Zaire, Angola and Zambia to South West Africa and Rhodesia, as the autosomal karyotype has apparently not changed structurally from one end to the other. Only the morphology of the Y chromosome has changed as mentioned earlier in the chapter. The 2n = 32 chromosome group from the Central African Republic also produced a 2n = 34 chromosome hybrid when mated to the 2n = 36 chromosome Mastomys from South Africa (Dickson, 1967). Mastomys in the intervening territory in which karyotypic analyses have not been done, may have undergone subsequent karyotypic change. Chromosome studies in this area would be very rewarding.

6.6 Behavioural and Other Differences in Mastomys with Different Chromosome Complements

The aggressiveness and frenzied activity displayed by the 2n = 32 chromosome group when compared with the 2n = 36 chromosome group has been noted in Chapters 4 and 5. More detailed and long term observations on the behaviour, habitats and habits of the different Mastomys groups are

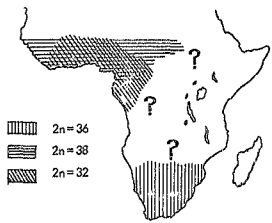


Fig. 54. Provisional geographic distribution of the three forms of Mastomys (from Matthey, 1966a).

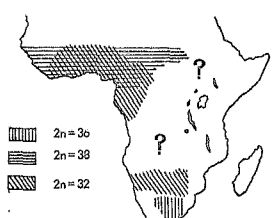


Fig. 55. Provisional geographic distribution of the three forms of Mastomys, showing new range of distribution in southern Africa, resulting from the present investigation.

necessary before these aspects of their characters could be used as identifying criteria. Nevertheless, the hyperactivit, and aggressive behaviour of the southern African 2n = 32 chromosome group when compared with the 2n = 36 chromosome group, is most noticeable after handling the two types for any length of time, to the extent that their chromosome numbers are often predictable.

Since these observations were made on Mastomys forms in southern Africa, Bellier (1975) in an abstract of his work on Mastomys in West Africa, mainly the Ivory Coast, noted that the 2n = 32 chromosome type was more aggressive than the 2n = 38 chromosome type in that area. Bellier's observations also indicated that those Mastomys captured in houses or villages had a chromosome number of 32, whereas those from the savanna, in forests or in cultivated or abandoned plantations had a chromosome number of 38. This indicates that their ecological niche and habits also differ considerably.

In southern Africa the 2n = 36 and the 2n = 32 chromosome forms are both found in fairly close association with human dwellings and farming activity, so there is no marked difference in their habits.

The 2n = 32 chromosome Mastomys from southern Africa observed in the present study appear to have more slender snouts than the 2n = 36 chromosome mice. This suggests that there may be a possible morphological difference in the cranial measurements of the two different groups.

Setzer (1975, and personal communication) has found two separate groups on morphological grounds within a Praomys (Mastomys) natalensis population from the Mkuze area of northern Natal. In one group the rostrum was short and relatively broad while in the other group " the rostrum is more

nearly in proportion". Presumably the latter is a longer and relatively narrower rostrum. This could represent an area where the two different chromosome groups overlap; on the other hand Setzer does feel that two kinds of Mastomys were also obtained from the same trap line at other localities sampled. He does not mention where these areas are. Differences in cranial measurements have also been found by Bellier (1975) in West African Mastomys.

6.7 Chromosome Rearrangements and Speciation

The karyotype differences exhibited by the Mastomys in this investigation (see Chapter 3), considered together with the fact that these two different groups, those with 2n = 32 and 2n = 36 chromosomes, cannot breed beyond the first generation are indicative of the groups being sufficiently isolated to be considered separate species, or in the last stages of speciation. Further investigation of the behavioural patterns within the two groups and their cranial measurements may further substantiate this separation.

The behaviour of the chromosomes of the hybrid at meiosis as presented in Chapter 5 suggests that chromosome rearrangements were instrumental in causing a post-mating isolating mechanism to operate between the 2n = 32 and 2n = 36 chromosome groups. It is possible, however, that in their natural environment these different chromosome groups do not come into contact with each other, i.e. they are allopatric, in which case they are already geographically isolated. If there is a zone of contact, i.e. the species are parapatric, there may be ethological isolating mechanisms which break down in the unnatural environment of breeding cages. As Mayr (1970)

has pointed out, "ethological isolation is the result of an interaction between external stimuli and the totality of internal drives. If no appropriate sex partner is available, internal drives continue to build up until readiness for mating can be induced even by highly inadequate stimuli". In either of these events, the chromosome rearrangements responsible for causing breakdown of meiosis could have occurred after the isolation of the new species or group, and thus not be directly implicated in the speciation process.

The distinct possibility remains, however, that the mechanical failure of the chromosomes to pair during meiosis could be the primary factor causing separation of the two groups. The mechanical failure of the chromosomes to pair would be a direct result of the chromosomal rearrangements, as is discussed in Chapter 5. In the past, chromosome rearrangements have not received much attention as potential isolating mechanisms, but White (1973) has suggested that rearrangements may on occasion, play a direct rôle, as divisive agents in speciation, generating genetic isolating mechanisms involving hybrid sterility.

The finding of naturally occurring hybrids (or lack of them) in possible zones of contact between the 2n = 32 and 2n = 36 chromosome group would help to clarify the situation.

Meanwhile one can only speculate on the evolutionary pattern of the 2n = 32 and 2n = 36 chromosome Mastomys, where the 2n = 36 chromosome population is confined to a relatively small area in South Africa and the 2n = 32 chromosome group possibly extends from the northern limit of the 2n = 36 chromosome group northwards to West Africa where it occurs

together with the 2n = 38 chromosome Praomys (Mastomys) erythroleucus.

Did the 2n = 36 chromosome group become geographically isolated from the rest? As Matthey (1973) has pointed out, there is a strong trend to accumulate the greatest possible number of centric fusions and consequently minimal diploid numbers in the course of evolution. He suggested that P. (Mastomys) erythroleucus with 2n = 38 chromosomes is the more primitive type while Mastomys with 2n = 32 chromosomes is the more evolved type (Matthey, 1966a). It does not seem likely then that there was geographic isolation of the southern group and subsequent increase in chromosome number. Geographic isolation, especially of peripheral populations, is however the most widely accepted pattern of speciation (Mayr, 1963, 1970).

With a higher chromosome group to the north and south it is easier to envisage a common ancestor with a higher chromosome number and rearrangements occurring within this range and spreading through the existing population leaving a group at either end, which underwent rearrangements of their own. White (1968) has proposed a stasipatric model of speciation, based on the geographic spread of chromosomal rearrangements through territory already occupied by the species, derived from his studies of morabine grasshoppers. In this type of speciation the ancestral groups are peripheral with derived karyotypes central. Mastomys are possibly in a similar situation.

There is also the possibility of an ancestral polymorphic population resulting from Robertsonian fusions, being divided into separate units or species by a genetic isolating mechanism. There also seems less likelihood

of this as no populations with 2n = 34 chromosomes have been discovered as yet so it would appear that the two obvious chromosome rearrangements, the Robertsonian fusion and the translocation were spread simultaneously.

6.8 Cytotaxonomy of Mastomys in Relation to Disease

The importance of cytogenetic and cytotaxonomic studies in the evolution and classification of the multimammate mouse Praomys (Mastomys) natalensis has been evident in the preceding sections of this chapter.

Since Chapter 1 was written, P. (Mastomys) natalensis as a taxonomic problem has come to the fore in just such a manner as had been intimated in that Chapter. The recent emergence of Lassa fever as an important virus disease has focussed attention on Mastomys. The basic ecology of Lassa fever in man appears to involve enzootic transmission of the virus in commensal populations of the single murine species Mastomys natalensis in West and possibly Central Africa (Monath, 1975). Monath recognises that the taxonomic status of Mastomys will require careful investigation in view of the two distinct forms of Mastomys in that area, especially as most arena viruses (of which Lassa virus is one) are associated with a single host species (Monath, 1975). It is unknown what role Mastomys plays in the Lassa fever cycle, be it a primary reservoir host, transmission bridge only, or merely a concomitant victim (Isaacson, 1975).

The importance of Mastomys in virus disease transmission cycles is also shown by the recent recovery of a virus closely related immunologically to Lassa virus from tissues of wild Praomys (Mastomys) natalensis captured in Mozambique (Wkly epidem. Rec., 1976). This locality is not far from

the area investigated in this study and therefore emphasizes the importance of the taxonomic study not only in southern Africa, but Africa as a whole. It is possible that the different forms of Mastomys may vary in their susceptibility to Lassa virus and other viral and also bacterial diseases.

ADDENDUM

During the final preparation of this dissertation six Praomys (Mastomys) natalensis from Natal were donated by Mr. K. Willan of the Zoology Department, University of Natal. A male and female were collected at Baynesfield (map reference 2930 Cb) and three males and a female at Pietermaritzburg (map reference 2930 Cb). These sites are about 70 kilometres from Durban, the type locality of Praomys (Mastomys) natalenis.

Chromosome counts of mitotic metaphase plates in bone marrow suspensions from these mice showed that the modal number was 32, the same as those Mastomys from the northern and eastern Transvaal, South West Africa and Rhodesia. Karyotypes constructed from photomicrographs of metaphase plates showed that the karyotypes of these Natal Mastomys were similar to the karyotypes of the northern and eastern Transvaal and South West Africa specimens, viz. :-

7 pairs of submetacentric chromosomes

5 pairs of metacentric chromosomes

3 pairs of acrocentric chromosomes

plus the two sex chromosomes. The X chromosomes were also large metacentric chromosomes and the Y was a large acrocentric chromosome, the same as described for the northern and eastern Transvaal and South West African Mastomys. In meiotic metaphase spreads there were 16 bivalents, and the XY bivalent was seen to have part side by side association in some plates and terminal association in others.

The finding of 2n = 32 Mastomys in Natal extends the range of this form further south east than previously thought. Setzer (personal communication) found two morphologically different forms of Mastomys at Mkuze in northern Natal. A more localised survey of Mastomys in Natal is necessary to ascertain whether the two forms i.e. 2n = 32 and 2n = 36, are possibly sympatric or parapatric in Natal. If 2n = 36 chromosome specimens are not found near Durban, the type locality of the species, then the 2n = 32 form would have prior claim to the species name of natalensis, and the 2n = 36 group would have to revert to one of the earlier species names used for that group of Mastomys.

REFERENCES

Balinsk,, B.I. (1960) An Introduction to Embryology. Philadelphia: W.B. Saunders Company.

Baltazard, M. (1963) Étude systematique d'un 'mesofoyer' de peste sauvage au Kurdistan Iranian. 1. Introduction. Bull. Soc. Path. exot., 56 : 1129-1141.

Bellier, L. (1975) The genus Mastomys in the Ivory Coast. Bull. Wld Hlth Org., 52 : 665.

Benirschke, K. (1967) Sterility and Fertility of Interspecific Mammalian Hybrids. In: Comparative Aspects of Reproductive Failure. Ed. K. Benirschke. New York : Springer - Verlag.

Benirschke, K. and Sullivan, M.M. (1966) Corpora lutea in proven mules. Fert. Steril., 17 : 24-33.

Bullough, W.S. (1951) Vertebrate Sexual Cycles. London : Methuen and Co. Ltd.

Clendenin, T.M. (1969) Intraperitoneal colchicine and hypotonic KCl for enhancement of abundance and quality of meiotic chromosome spreads from hamster testes. Stain Technol., 44 : 63-69.

Coetzee, C.G. (1967) The breeding season and population structure of the multimammate mouse Praomys (Mastomys) natalensis (A. Smith, 1834) in the Transvaal highveld. M. Sc. Thesis, University of Pretoria.

Craft, W.A. (1938) The sex ratio in mules and other hybrid mammals. Q. Rev. Biol., 13 : 19-40.

Davis, D.H.S. (1948) Sylvatic plague in South Africa: History of plague in man, 1919-43. Ann. trop. Med ., 42 : 207-217.

Davis, D.H.S. (1962) Distribution patterns of southern African Muridae with notes on some of their fossil antecedents. Ann. Cape prov. Mus., 2 : 56-76.

Davis, D.H.S. (1963) Wild rodents as laboratory animals and their contribution to Medical Research in South Africa. S. Afr. J. med. Sci., 28 : 53-69.

Davis, D.H.S. (1965) Classification problems of African Muridae. Zool. Afr., 1 : 121-145.

Davis, D.H.S. and Oettlé, A.G. (1958) The multimammate mouse, Rattus (Mastomys) natalensis Smith: A laboratory adapted African wild rodent. Proc. zool. Soc. Lond., 131 : 293-299.

De Wit, C. (1972) An ecological study of a small mammal community with emphasis on the status of Praomys (Mastomys) natalensis. M. Sc. Thesis, University of Pretoria.

Dickson, J.M. (1967) An investigation into the possibility of obtaining hybrids from cross breeding different Praomys (Mastomys) forms and a chromosome analysis of the hybrids obtained. Unpublished Report.

Ellerman, J.R. (1941) Families and Genera of Living Rodents. Vol. II. London : British Museum (Nat. Hist.).

Ellerman, J.R., Morrison-Scott, T.C.S. and Hayman, R.W. (1953) Southern African mammals 1758-1951 : A reclassification. London : British Museum (Nat. Hist.).

Evans, E.P., Breckon, G. and Ford, C.E. (1964) An air-drying method for meiotic preparations from mammalian testes. Cytogenetics, 3 : 289-294.

Ford, C.E. and Hamerton, J.L. (1956a) A colchicine, hypotonic citrate squash sequence for mammalian chromosomes. Stain Technol., 31 : 247-251.

Ford, C.E. and Hamerton, J.L. (1956b) Chromosomes of five Rodent species. Nature, Lond., 177 : 140-141.

Ford, E.H.R. (1973) Human Chromosomes. London : Academic Press.

Hamerton, J.L. (1958) Mammalian Sex Chromosomes. In: Symposium on Nuclear Sex. Ed. D.R. Smith and W.M. Davidson. London : Heinemann.

Hsu, T.C. (1952) Mammalian chromosomes in vitro. 1. The Karyotype of Man. J. Hered., 43 : 167-172.

Hsu, T.C. and Mead, R.A. (1969) Mechanisms of Chromosome Changes in Mammalian Speciation. In: Comparative Mammalian Cytogenetics. Ed. K. Benirschke. New York : Springer-Verlag.

Huang, C.C. (1968) Karyologic and autoradiographic studies of the chromosomes of Rattus (Mastomys) natalensis. Cytogenetics, 7 : 97-107

Huang, C.C. and Strong, L.C. (1962) Chromosomes of the African mouse. J. Hered., 52 : 95-99.

Hughes, A. (1952) Some effects of abnormal tonicity on dividing cells in chick tissue cultures. Q. Jl Microsc. Sci., 93 : 207-219.

Hungerford, D.A. (1965) Leucocytes cultured from small inocula of whole blood and the preparation of metaphase chromosomes by treatment with hypotonic KCl . Stain Technol., 40 : 333-338.

Isaäcson, M. (1975) The ecology of Praomys (Mastomys) natalensis in southern Africa. Bull. Wld Hlth Org., 52 : 629-636.

King, J.M. (1967) The sterility of two rare Equine hybrids. In: Comparative Aspects of Reproductive Failure. Ed. K. Benirschke. New York : Springer-Verlag.

Lay, D.M. and Nadler, C.F. (1969) Hybridization in the rodent genus Meriones. 1. Breeding and cytological analyses of Meriones shawi (♀) X Meriones libycus (♂) hybrids. Cytogenetics, 8 : 35-50.

Lundholm, B.G. (1955) Remarks on some South African Murinae. Ann. Transv. Mus., 22 : 321-329.

Makino, S. and Nishimura, I. (1952) Water pretreatment squash technique. A new and simple practical method for the chromosome study of animals. Stain Technol., 27 : 1-7.

Matthey, R. (1945) L'évolution de la formule chromosomiale chez les vertébrés. Experientia, 1 : 50-56, 78-86.

Matthey, R. (1954) Nouvelles recherches sur les chromosomes des Muridae. Caryologia, 6 : 1-44.

Matthey, R. (1955) Nouveaux documents sur les chromosomes des Muridae. Problèmes de cytologie comparée et de taxonomie chez les Microtinae. Rev. suisse Zool., 62 : 163-206.

Matthey, R. (1957) Cytologie et taxonomie du genre Meriones, Illiger (Rodentia - Muridae - Gerbillinae). Säugetierk. Mitt., 5 : 145-150.

Matthey, R. (1958) Les chromosomes et la position systématique de quelques Murinae africains (Mammalia - Rodentia). Acta trop., 15 : 97-117.

Matthey, R. (1965a) Cytogenetic Mechanisms and Speciations of Mammals. In: The Chromosome - Structural and Functional Aspects. In Vitro, 1 : 1-11.

Matthey, R. (1965b) *Etudes de cytogénétique sur des Murinae africains* appartenant aux genres Arvicanthis, Praomys, Acomys et Mastomys (Rodentia). Mammalia, 29 : 228-249.

Matthey, R. (1966a) Cytogénétique et taxonomie des Rats appartenant au sous-genre Mastomys Thomas (Rodentia - Muridae). Mammalia, 30 : 105-119.

Matthey, R. (1966b) Une inversion péricentrique à l'origine d'un polymorphisme chromosomique non-Robertsonien dans une population de Mastomys (Rodentia - Murinae). Chromosoma, 18 : 188-200.

Matthey, R. (1973) The Chromosome Formulae of Eutherian Mammals. In: Cytotaxonomy and Vertebrate Evolution. Ed. A.B. Chiarelli and E. Capanna. London : Academic Press.

Mayr, E. (1963) Animal Species and Evolution. Cambridge : Harvard University Press.

Mayr, E. (1970) Populations, Species and Evolution. Cambridge : Harvard University Press.

Meester, J. (1964) An interim classification of southern African mammals. The Zoological Society of Southern Africa and The S.A. Council for Scientific and Industrial Research (stencil).

Misonne, X. (1968) Rodentia : main text. In: Smithsonian Institution preliminary identification Manual for African Mammals, 19 : 1-55. Ed. J. Meester.

Monath, T.P. (1975) Lassa Fever: review of epidemiology and epizootiology. Bull. Wld Hlth Org., 52 : 577-592.

Moorehead, P.S., Nowell, P.C., Melman, W.J., Battips, D.M. and Hungerford, D.A. (1960) Chromosome preparations of leucocytes cultured from human peripheral blood. Expl Cell Res., 20 : 613-616.

Nadler, C.F. (1969) Chromosome Evolution in Rodents. In: Comparative Mammalian Cytogenetics. Ed. K. Benirschke. New York : Springer-Verlag.

Nalbandov, A.V. (1958) Reproductive Physiology. San Francisco : W.H. Freeman and Co.

Nevo, E. (1969) Mole Rat Spalax ehrenbergi: mating behavior and its evolutionary significance. Science, 163 : 484-486.

Ohno, S., Becak, W. and Becak, M. (1964) X-autosome ratio and the behaviour pattern of individual X chromosomes in placental mammals. Chromosoma, 15 : 14-30.

Oliff, W.D. (1953) Mortality, fecundity and intrinsic rate of natural increase of the multimammate mouse, Rattus (Mastomys) natalensis (Smith) in the laboratory. J. Anim. Ecol., 22 : 217-226.

Petter, F. (1957) Remarques sur la systematique des Rattus africains et description d'une forme nouvelle de l'Air. Mammalia, 21 : 125-132

Roberts, A. (1944) Andrew Smith's early descriptions of animals. Bull. S. Afr. Mus. Ass., 3 : 238-241.

Roberts, A. (1951) The Mammals of South Africa. Johannesburg : Central News Agency.

Robertson, W.R.B. (1916) Chromosome studies. 1. Taxonomic relationships shown in the chromosomes of Tettigidae and Acrididae. V-shaped chromosomes and their significance in Acrididae, Locustidae and Gryllidae : chromosomes and variation. J. Morph., 27 : 179-331.

Rothfels, K.H. and Siminovitch, L. (1958) An airdrying technique for flattening chromosomes in mammalian cells grown in vitro. Stain Technol., 33 : 73-77.

Sachs, L. (1952) Polyploid evolution and mammalian chromosomes. Heredity, Lond., 6 : 357-364.

Setzer, H.W. (1956) Mammals of the Anglo-Egyptian Sudan. Proc. U.S. natn. Mus., 106 : 447-587.

Setzer, H.W. (1975) Discussion in: Recent Isolations of Lassa virus from Nigerian rodents. Bull. Wld Hlth Org., 52 : 613.

Tobias, P.V. (1953) Trends in the evolution of mammalian chromosomes. S. Afr. J. Sci., 50 : 134-140.

Tobias, P.V. (1956) Chromosomes, Sex Cells and Evolution in a Mammal, based mainly on studies of the reproductive glands of the Gerbil and a new list of Chromosomes of Mammals. London : Lund Humphries.

Vorontsov, N.N. (1973) The Evolution of the Sex Chromosomes. In: Cytotaxonomy and Vertebrate Evolution. Ed. A.B. Chiarelli and E. Capanna. London : Academic Press.

Wahrman, J., Goitein, R. and Nevo, E. (1969a) Chromosome Forms in a Subterranean Rodent. In: Comparative Mammalian Cytogenetics. Ed. K. Benirschke. New York : Springer - Verlag.

Wahrman, J., Goitein, R. and Nevo, E. (1969b) Mole Rat Spalax: evolutionary significance of chromosome variation. Science, 164 : 82-84.

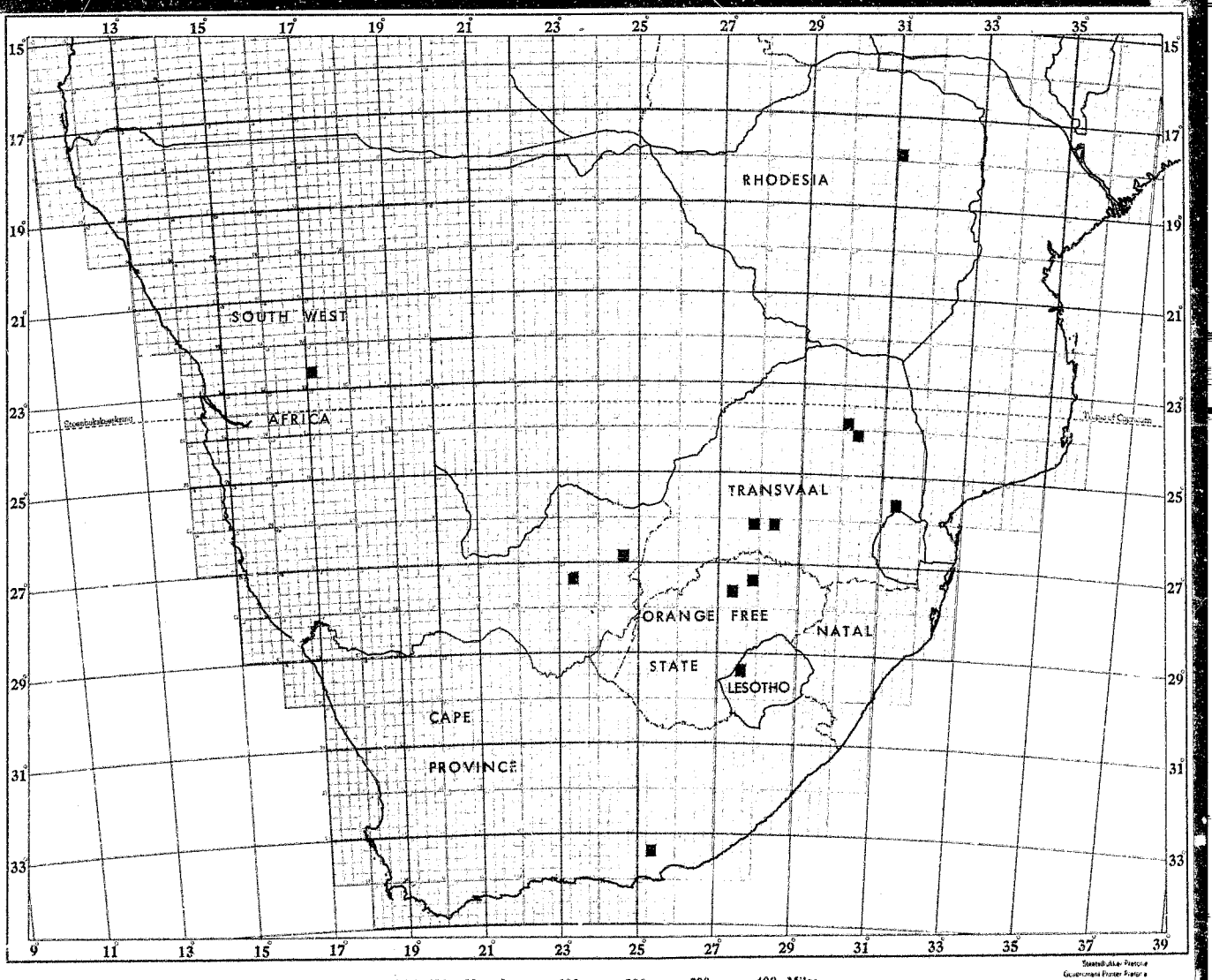
Wallace, C. and Fairall, N. (1967) Chromosome polymorphism in the impala. S. Afr. J. Sci., 63 : 482-486.

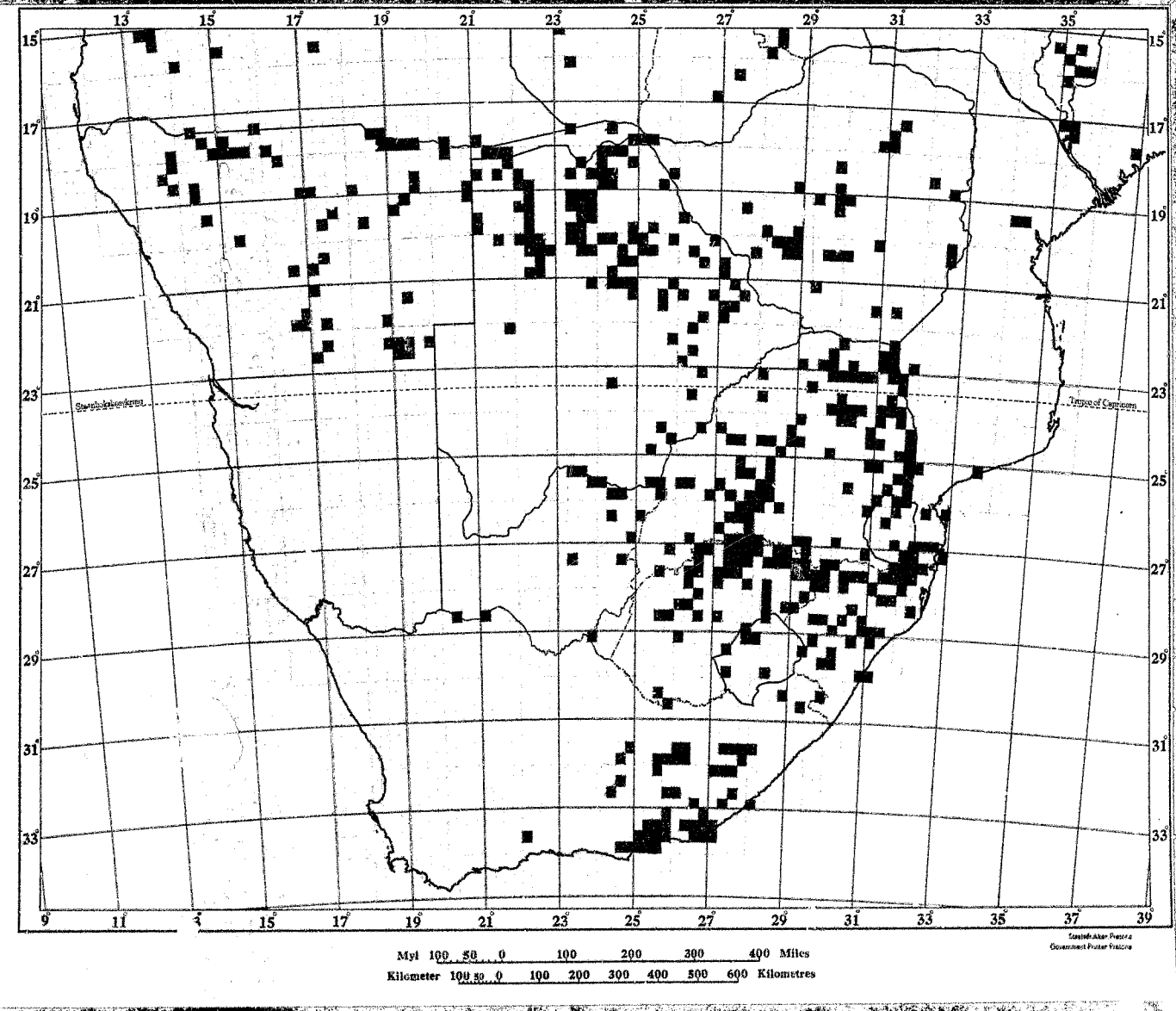
White, M.J.C (1968) Models of speciation. Science, 159 : 1065-1070.

White, M.J.D. Animal Cytology and Evolution. 3rd ed. London: Cambridge University Press.

Wkly epidem. Rec., (1976) Lassa Fever Surveillance. 20 Feb. : 66

Zahavi, A. and Wahrman, J. (1957) The cytotaxonomy, ecology and evolution of the gerbils and jirds of Israel (Rodentia : Gerbillinae). Mammalia, 21 : 341-380.


RHODESIA
SOUTH WEST
AFRICA
TRANSVAAL
ORANGE FREE
STATE
NATAL
LESOTHO
CAPE
PROVINCE
Myl 100 50 0 100 200 300 400 Miles
Kilometer 100 50 0 100 200 300 400 500 600 Kilometres


13° 15° 17° 19° 21° 23° 25° 27° 29° 31° 33° 35°
15° 17° 19° 21° 23° 25° 27° 29° 31° 33°
9° 11° 13° 15° 17° 19° 21° 23° 25° 27° 29° 31° 33° 35° 37° 39°
Tropic of Capricorn
Myl 100 50 0 100 200 300 400 Miles
Kilometer 100 50 0 100 200 300 400 500 600 Kilometres

Author Hallett Janet Mary
Name of thesis Cytological and cytogenetical studies on the multimammate mouse, Praomys (mastomys) Natalensis. 1977

PUBLISHER:
University of the Witwatersrand, Johannesburg
©2013

LEGAL NOTICES:

Copyright Notice: All materials on the University of the Witwatersrand, Johannesburg Library website are protected by South African copyright law and may not be distributed, transmitted, displayed, or otherwise published in any format, without the prior written permission of the copyright owner.

Disclaimer and Terms of Use: Provided that you maintain all copyright and other notices contained therein, you may download material (one machine readable copy and one print copy per page) for your personal and/or educational non-commercial use only.

The University of the Witwatersrand, Johannesburg, is not responsible for any errors or omissions and excludes any and all liability for any errors in or omissions from the information on the Library website.