Supplement to TOBIAS, P.V. Cytological and cytogenetical studies on the testis of the gerbil. p. 366.

South African Journal of Science

Suid-Afrikaanse Joernaal van Wetenskap

The QL 737. R6

25550

PUBLISHED BY THE SOUTH AFRICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

UITGEGEE DEUR DIE SUID-AFRIKAANSE GENOOTSKAP VIR DIE BEVORDERING VAN WETENSKAP

JUNIE 1952 JUNE

Deel/Vol. 48 No. 11

Contents - Inhoud

RESEARCH IN INDUSTRY 337

THE HEAVENS IN JULY 338

THE BEDFORD VIEW-EDENVALE AREA: A LAND UTILIZATION SURVEY ... *Dr Monica M. Cole* 339

ON THE CRANIAL MORPHOLOGY OF AMBYSTOMA MACULATUM (SHAW) ... *Mr J. G. Theron* 343

THE CHROMOSOMAL COMPLEMENT OF THE GERBIL, TATERA BRANTSII DRACO ... *Dr P. V. Tobias* 366

A NEW JOURNAL OF ACOUSTICS ... 373

VEGETATION PATTERNS REVEALING FORMER KALAHARI DUNE-SANDS IN SOUTHERN RHODESIA ... ... *Prof. Lester King* 374

SOUTH AFRICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE—ANNUAL MEETING, CAPETOWN, JULY 1952... 316

REVIEWS—

Radio 377, 378, 380

Ceylon 377

Horses 377

Mathematics 378

Geology 378

Steel 379

Thermodynamics 379

Organic Chemistry 380

Botany 380

Psychology 380

Acoustics 381

Tribe under Trust 381

RECENT BOOKS RECEIVED 381

Free to Members
Kosteloos aan Lede

Price 3/6 Prys

Registered at the G.P.O. as a newspaper

37/6 per annum (post free)
37/6 per jaar (pos vry)

THE CHROMOSOMAL COMPLEMENT OF THE GERBIL, TATERA BRANTSII DRACO

P. V. Tobias
Department of Anatomy,
University of the Witwatersrand, Johannesburg

ABSTRACT

The chromosomes of dividing germ-cells in the gerbil are characterized. The diploid chromosomal number of *Tatera brantsii draco* is 34 and the haploid number 17. There are 16 homologous pairs of chromosomes, 5 with sub-median constrictions and 11 with sub-terminal constrictions. The two largest chromosomes constitute a heteromorphic pair and each member has one primary and two secondary constrictions; these unequal chromosomes are the presumptive sex-chromosomes. The results are represented graphically in a typegraph of the species which may assist in explaining the evolutionary divergence of the different Tatera species.

INTRODUCTION

MAMMALIAN chromosomes are difficult to characterize because they are extremely short and numerous. In an earlier paper (1947), it was suggested that one method of surmounting these difficulties was by characterizing the chromosomes in groups and representing them graphically.

The present paper places on record a new application of these techniques to a species of the Gerbillinae.

The African gerbils of the genus *Tatera* fall into two species-groups, the Afra and the Robusta groups (Davis, 1949), based on their morphological features and habitat. Each group, in turn, contains a number of species and sub-species :—

Robusta :

1. *T. robusta* (13 sub-species).
2. *T. nigricauda* (5 sub-species).
3. *T. guineae* (2 sub-species).
4. *T. schinzi* (21 sub-species).

Afra :

1. *T. afra* (2 sub-species).
2. *T. brantsii* (12 sub-species).
3. *T. valida* (11 sub-species).
4. *T. giffardi* (7 sub-species).
5. *T. leucogaster* (5 sub-species).
6. *T. ruddi* (monotypic).

Both groups are represented in South Africa by 'good' species, Robusta by *T. schinzi* and the Afra group by *T. afra*, *T. brantsii* and *T. ruddi*.

Geographically, *T. afra* is isolated from the other species, being confined to the coastal plains of the western Cape Province. *T. ruddi* is known only from its type locality in Zululand. The other two species, *T. brantsii* and *T. schinzi*, overlap in their distribution. *T. brantsii*, according to Davis, ranges over the highveld of the eastern and north-western Cape Province, Orange Free State, the southern Transvaal, Natal and Zululand, the Kalahari from Kuruman in the south to Ovamboland and southern Angola, northern Bechuanaland and Barotseland (Northern Rhodesia). Its range thus includes the Witwatersrand area in which the animals used in the present study were trapped. *T. schinzi* occurs in the Kalahari and its fringes in the north-west Cape and western Orange Free State, the bushveld and the lowveld of the northern Transvaal and, west to east across the sub-continent from southern Angola, through Northern Rhodesia, to Nyasaland and Moçambique.

The ranges of *T. schinzi* and *T. brantsii* overlap in and on the fringes of the Kalahari. The presence of these two forms in the same locality directs attention to their evolutionary divergence. On current views, it must be assumed that, at some time in the past, the once actually or potentially interbreeding array of *Tatera* stock became segregated into groups of populations reproductively isolated from one another.

Reproductive isolation was probably preceded by geographical and ecological isolation, which thus enabled the various population groups to evolve further. Among the features of this divergence must have been some which ensured reproductive isolation, even when spatial barriers were broken down and the ranges of the population groups once more overlapped.

Possible isolating mechanisms include non-coincidence of the breeding season, difference of habitat and, non-correspondence of the chromosomal complements, resulting in the breakdown of meiosis and consequent hybrid sterility. Measroch (1948) has shown that *T. brantsii* and *T. schinzi* breed all the year round, although periods of maximum and minimum breeding occur. Thus, the reproductive isolation is not attributable to differences in the breeding season.

Differences in choice of habitat, food and burrowing habits have been noted (Roberts, 1936; Davis, 1949) and Davis speaks of the *T. brantsii* forms as primarily 'plains' gerbils and *T. schinzi* forms as 'savanna and woodland' gerbils.

In addition, it is possible that there is a chromosomal basis for the absence of a hybrid population. A comparison of the chromosomes of various species of *Tatera*—to which it is hoped the present study will lead—may settle this point.

MATERIAL AND METHODS

The animals were trapped by the field staff of the Plague Research Laboratory, in the vicinity of Johannesburg. This area lies within the geographical range of the *draco* sub-species of *Tatera brantsii* and the animals were identified by Mr D. H. S. Davis, the Union Government Ecologist and Chief Rodent Officer, as members of this sub-species. One other sub-species, *T. brantsii brantsii*, occurs in South Africa, but it was not possible to procure any material from this animal for comparison.

The animals were trapped at varying intervals between July and October. Although all trapped males showed active spermatogenesis, a higher percentage of tubules was actively functional in those animals killed in the later months.

T. brantsii draco was selected for several reasons. Firstly, through the courtesy of Mr Davis, there was a ready supply of animals. Secondly, no other species of the sub-family, Gerbillinae, of the Rodents had previously been studied cytogenetically. Thirdly, the study might help in unravelling certain problems concerning the speciation of *Tatera*.

The testes of eight male animals and the ovaries of two female animals were removed under ether anaesthesia and, from these, sections, squash and smear preparations were made. Half of each testis and one ovary from each female animal were fixed in Bouin's picro-formol-acetic fixing fluid for 24 hours. The tissues were dehydrated, cleared and embedded in paraffin wax. Sections were cut at thicknesses of 10μ, 12μ and 16μ. The stains used were Heidenhain's iron haematoxylin, Ehrlich's acid haematoxylin, Newton's crystal violet and Feulgen's nucleal reagent (leuco - basic fuchsin).

Most of the second halves of the testes and the remaining ovaries were fixed in 25 per cent acetic acid in absolute alcohol. After 24 hours in the fixing fluid, the material was squashed in acetic orcein. The squash preparations, fixed by albumin on the cover-slips, were floated off the slides in acetic alcohol and re-stained with Ehrlich's acid haematoxylin, Heidenhain's iron haematoxylin or Feulgen's leuco-basic fuchsin and light green, in accordance with a method first suggested by Brenner (1947).

Finally, some testicular material, as well as pieces of liver from the same animals, were smeared and fixed in 95 per cent alcohol. These smears were then stained with Pappenheim's and Unna's methyl-green pyronin technique.

Observations were made using a 90x, 97x or 100x oil-immersion objective and a 10x, 12·5x, 15x or 20x eyepiece. Drawings were made with the aid of a Zeiss camera lucida (magnification factor 1·8x).

Measurements made directly on the preparations with an ocular micrometer can introduce a significant error. This was obviated by measuring the lengths of the chromosomes on camera lucida drawings, which were made at magnifications of 3250x (90x objective, 20x eyepiece) or 3500x (97x objective, 20x eyepiece). The final length was corrected to the first decimal place; it is assumed that errors of measurement or of drawing became insignificant when divided by the magnification. Difficulties of overlap or foreshortening were minimized by focussing with the fine adjustment. Where this was not possible, the plates were rejected.

OBSERVATIONS

Chromosomal number

The number of spermatogonial chromosomes in the gerbil is 34. In 15 squashed

spermatogonial pro-metaphasic and metaphasic nuclei, 34 chromosomes have been counted. The chromosomal number in spermatogonia is assumed to be the type-number of the species, i.e. the diploid number. Confirmation of this number is afforded by the presence of 17 paired threads or tetrads in several squashed pachy-diplotene nuclei.

Thus, the diploid number of chromosomes in the gerbil is 34 and the haploid number 17 (Figs. 1 and 2).

Chromosomal morphology

The two chromatids of each chromosome are clearly seen in many cells. Often this feature shows best at one end of the chromosome, where the paired tips may be rather widely separated. Occasionally the two chromatids of a pair appear coiled about each other one or more times (Fig. 1, Plate C ; Fig. 2, Plate D). This chromatid relational coiling is more easily seen in the gerbil than in the albino rat.

At pro-metaphase a loose coiling is seen in several chromosomes : according to Darlington (1935), such a 'relic spiral' represents the uncoiling internal spiral developed during the previous mitotic division.

As in the albino rat (Tobias, 1947), constrictions are most clearly seen in the pro-metaphasic chromosomes ; however, constrictions are much more clearly visible at metaphase in the gerbil than at metaphase in the rat. This may be due to longer constrictions or to a lesser degree of spiralization in the gerbil. 'Centromeric granules' have not been seen in the gerbil.

Most chromosomes possess only one distinct constriction, appearing as a short achromatic gap. In a few chromosomes, containing more than one constriction each, the anaphase configurations indicate which is the primary (centric) and which the secondary constriction. The positions of constrictions vary in different chromosomes, but, in general, constrictions may be grouped as sub-terminal or sub-median.

Of the sub-terminal constrictions, the two types described in the albino rat occur in the gerbil, namely those so close to the end of the chromosome that only a small chromatic granule is visible distal to the constriction, and those situated one-tenth to one-fifth of the chromosomal length away from the end. Intergrades occur between these two positions, and between the sub-terminal constrictions furthest from the end and the sub-median constrictions. Thus, in borderline situations, classification of constrictions as sub-terminal or sub-median is arbitrary. Nevertheless the numbers of sub-terminal and sub-median constrictions in different plates are fairly constant.

In the best plates (e.g. A, B and C), there are 22 chromosomes with sub- terminal and 12 with sub-median constrictions ; in a few plates the numbers are 20 and 14 respectively, one pair presumably being subject to two interpretations.

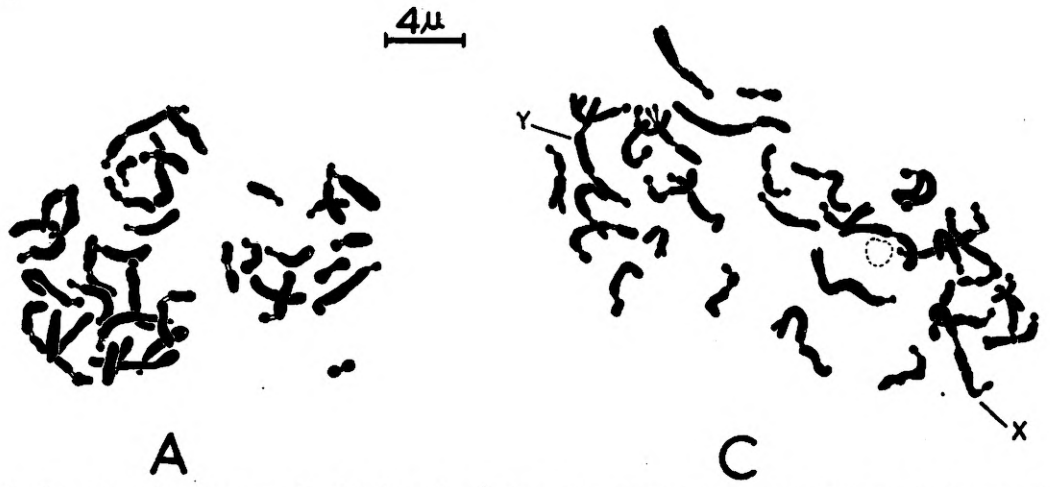

Fig. 1—Metaphase (A) and pro-metaphase (C) plates of spermatogonial nuclei : squash preparation stained with haematoxylin.

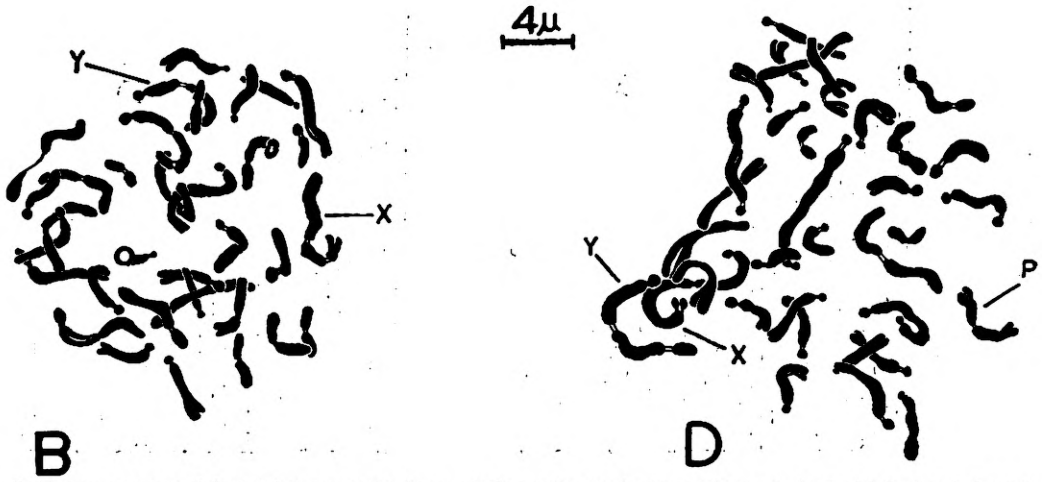

Fig. 2—Two spermatogonial nuclei at pro-metaphase: acetic orcein squash preparations restained with Feulgen's leuco-basic fuchsin. (Chromosome P belongs to a neighbouring plate.)

Chromosomal length and size-range

Graphs A, B, C and D represent the lengths of the chromosomes in the corresponding spermatogonial plates: the lengths are arranged in descending order. A comparison of the graphs indicates the size-range for chromosomes in any part of the series. By such a comparison, a composite graph has been drawn of the maximum and minimum lengths of the chromosomes at corresponding positions in the series (Fig. 4).

The 34 chromosomes of the gerbil range in length from 1·4–1·9 μ, the size range of the smallest, to 6·0–7·9 μ, the length of the largest chromosome. The longest chromosome in the gerbil is thus appreciably longer than the longest pair in the rat (3·6–4·4 μ). On the other hand, the shortest chromosome in the gerbil is not as small as the shortest pair in the rat (0·8–0·9 μ). All the intermediate chromosomes (Nos. 3–32) in the gerbil are grouped between 1·5 μ and 5·3 μ, as against the rather narrower distribution of the rat's chromosomes (pairs 2–19) between 0·8 μ and 3·9 μ.

As in the composite graph of the albino rat, the graph of the gerbil's chromosomes must be interpreted with discrimination, in view of possible variations in the linear order of chromosomes. The main conclusion to be drawn from the graph is the magnitude of the size-range for any part of the linear series.

The ranges vary in the chromosomal series, much as they do in the albino rat. The absolute value of the range is smallest in the six shortest chromosomes (Nos. 29–34), where its average value is 0·5μ. In the intermediate group (chromosomes 3–28), the average range is 0·7 μ, while in the longest pair (chromosomes 1–2), the size-range is greatest, averaging 1·6 μ. There is no marked exception, as in the albino rat where the X-chromosome, though one of the longer elements, has a very small range.

When the relative range is calculated by expressing the average range as a percentage of the chromosomal length, the intermediate and smaller groups of chromosomes have more or less the same relative ranges (approximately 24 per cent of minimal length or 19 per cent of maximal length). On the other hand, the relative range of the largest pair is 33 per cent. of minimal and 25 per cent. maximal length. The longest chromosomes therefore have greater size-ranges, both absolutely and relatively, than the others; there is no distinction in this regard between the intermediate and smaller groups.

The behaviour of the longest pair of chromosomes may be understood as the consequence of their tardy contraction; some metaphase plates have been fixed and stained before the complete contraction of these chromosomes, while others have been arrested after these chromosomes have attained their minimal length.

THE CHARACTERIZATION OF THE GERBIL'S CHROMOSOMES

We have considered several properties of the gerbil's chromosomes: their number,

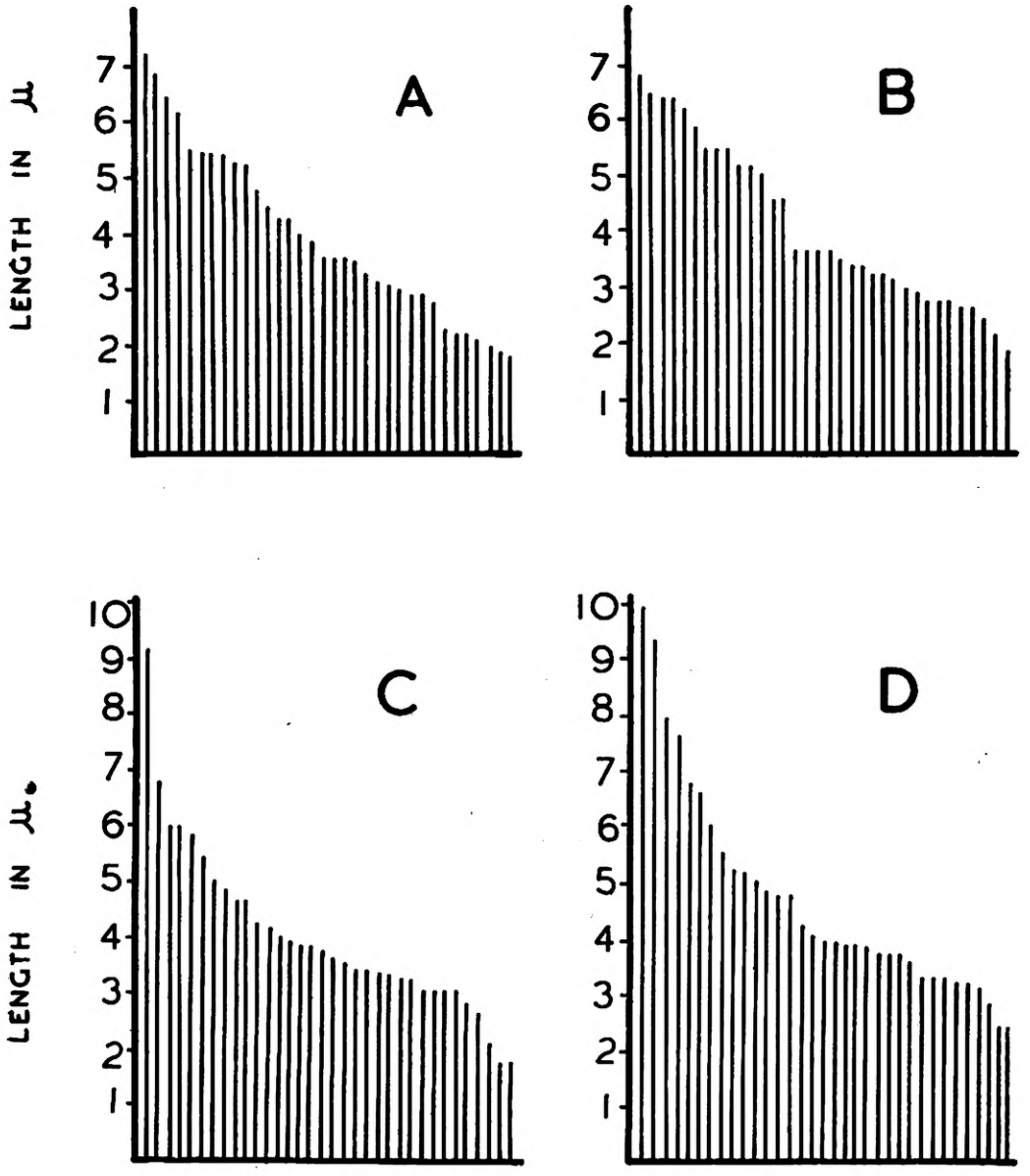

Fig. 3—Graphs of the chromosomal complements depicted in Figs. 1 & 2.

morphology, length and size-ranges. It remains to correlate these sets of information in order to characterize the chromosomal complement. By a close study of camera lucida drawings and measurements of the chromosomes, controlled by discriminative microscopic study of the plates, the entire complement of each individual plate was paired off and thus a number of isolated chromosomal complements were characterized. By comparing these individual characterizations, as well as by a study of the composite graph, the following species-characterization has been made.

The largest two chromosomes are readily distinguished by their size and morphology. They have a characteristic pattern of constrictions (see chromosomes X and Y in

Plates B, C and D). One constriction, consistently present, is sub-median in position; from studies on anaphase figures, it is clearly the centric constriction. In addition, each arm is divided into a longer, central segment and a shorter, distal or terminal segment by a secondary constriction. In one arm, this constriction is noticeably further from the extremity, thus cutting off a longer distal segment, than in the other arm. We have then a chromosome divided into two arms and each arm sub-divided into two segments, making four segments in all, delimited by three constrictions.

Although the two largest chromosomes both possess this pattern of constrictions, there are generally significant differences in length between them. Their size ranges do not even overlap, the range for the largest element being 6·0–7·9 μ, while that of the second largest is 4·6–6·0 μ.

On grounds of this inequality in length and, because the rest of the complement can be arranged in homomorphic pairs, it is inferred that the two largest chromosomes constitute a heteromorphic pair. In addition, these two chromosomes are not completely homologous, each to each, and, during meiosis, they exhibit many peculiar properties, characteristic of the sex-chromosomes

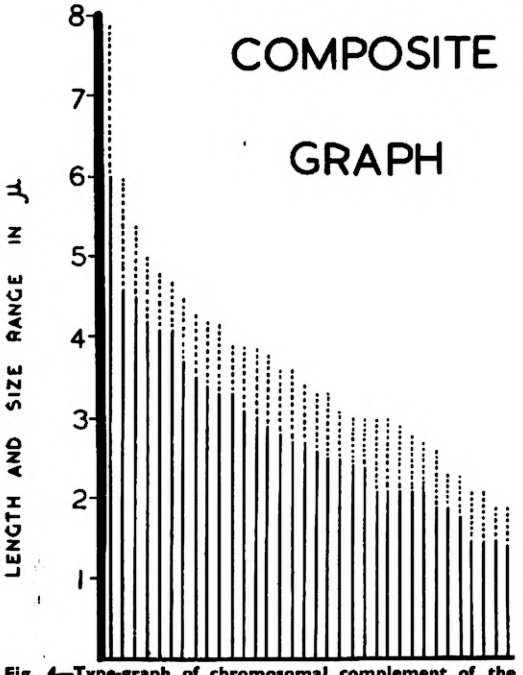

Fig. 4—Type-graph of chromosomal complement of the gerbil.

of other mammals. It is concluded, therefore, that these large heteromorphic chromosomes are the sex-chromosomes of the gerbil. It has not been possible to determine which of the two is the X-chromosome, i.e. the element which may be expected to occur twice in the diploid complement of female gerbils. However, in all mammals in which males and females have been studied so far, the X has been generally larger, occasionally equal to, but never smaller than the Y. By analogy with these other mammals, it may be accepted that the largest chromosome in the gerbil's complement is the presumptive X and the second largest the presumptive Y.

Both members of the second largest pair of chromosomes possess a sub-median centric constriction, while one arm usually contains a sub-terminal secondary constriction. The size-ranges for chromosomes 3 and 4 are 4·5–5·3 μ and 4·2–5·0 μ. Because of the considerable overlap between the two ranges, the size range for the pair is best expressed as the combined total range, i.e. 4·2–5·3 μ.

At the other end of the linear series, the two smallest chromosomes generally have sub-median constrictions and may be coupled as pair 17. Their size-range is :—

Chromosome No. 33 : 1·5–1·9 μ.
Chromosome No. 34 : 1·4–1·9 μ.
Pair No. 17 (combined range) : 1·4–1·9 μ.

In several plates, this pair with the sub-median constrictions is the second smallest in the complement, there being a pair with sub-terminal constrictions at the lower end of the series. Generally, however, the pair with sub-terminal constrictions is the 16th pair, having a combined size-range of 1·5–2·1 μ.

Between the two largest and the two smallest pairs are 13 pairs of chromosomes. They lie in that part of the length-graph where there is a relative flattening (see composite graph) and, therefore, it is more difficult to pair them off accurately.

The third largest pair of chromosomes with sub-median constrictions may be recognized without much difficulty. It may lie third or fourth in the linear series of pairs and its combined size-range in metaphase plates is 4·1–4·8 μ.

The preceding pair sometimes exchanges positions with the largest pair of chromosomes containing a sub-terminal constriction. The latter pair, No. 4, has a combined size-range in metaphase plates of 3·5–4·6 μ.

Pairs 5 and 6, again, are interchangeable in the linear series; one pair has sub-terminal, the other sub-median, constrictions. The size-range of the pair with sub-terminal constrictions (the second largest pair in the complement with constrictions in this position) is 3·3–4·0 μ; while the range for the other pair, namely that with sub-median constrictions, is 3·4–4·1 μ. The latter will therefore be designated pair 5 and the former pair 6.

Then follows a group of four successive pairs (Nos. 7–10) with sub-terminal constrictions. The combined size-range of these four pairs is 2·5–3·9 μ and, they are characterized in a group. The method of group-characterization, as tested on the rat, best overcomes such difficulties as the variable linear order of chromosomes and the arbitrary classification of constrictions as sub-terminal or sub-median.

The 11th pair of chromosomes possesses a sub-median constriction in almost every plate and a size-range of 2·3–2·7 μ. Its members constitute the second smallest pair of chromosomes with sub-median constrictions.

The 12th to 15th pairs have sub-terminal constrictions. In several plates, one of these pairs (No. 14 or 15) has been described as possessing a sub-median constriction, for reasons already indicated. The size-range for this group of four pairs is 1·8–3·0 μ.

The 16th and 17th pairs have already been characterized.

CONCLUSION

Arising from our composite graph and from characterizations of nine individual spermatogonial plates, we may now summarize the composite species-characterization as in Table 1.

In the above account of the chromosomal complement of the gerbil, attention has been drawn to a number of contrasts between the gerbil and the albino rat. Table 2 summarizes the salient differences between the two species.

ACKNOWLEDGMENTS

I am grateful to Professor R. A. Dart for having provided facilities in the Anatomy Department for this investigation. My thanks are due to Professor J. Gillman for his advice and guidance during the course of the study and for his valued criticisms of the draft report.

This work was made possible, at the outset, by a C.S.I.R. Research Grant and, later in the study, by the award of a Rusterholz Memorial Scholarship.

TABLE 1

Pair or group of chromosomes	Length	Position of centric constriction
Pair 1 X ...	6·0—7·9μ	Sub-median
Y ...	4·6—6·0μ	
Pair 2	4·2—5·3μ	Sub-median
Pair 3	4·1—4·8μ	Sub-median
Pair 4	3·5—4·6μ	Sub-terminal
Pair 5	3·4—4·1μ	Sub-median
Pair 6	3·3—4·0μ	Sub-terminal
Pairs 7-10 ...	2·5—3·9μ	Sub-terminal
Pair 11 ...	2·3—2·7μ	Sub-median
Pairs 12-15 ...	1·8—3·0μ	Sub-terminal
Pair 16 ...	1·5—2·1μ	Sub-terminal
Pair 17 ...	1·4—1·9μ	Sub-median

TABLE 2

Feature	Albino rat	Gerbil
Chromosomal number ...	42	34
Number of chromosomes with sub - terminal constrictions	28 (or 66·7%)	22 (or 64·7%)
Number of chromosomes with sub - median constrictions	14 (or 33·3%)	12 (or 35·3%)
Number of chromosomal arms	56	48
Position of *X* in linear series ...	5th	1st
Position of *Y* in linear series ...	34th	2nd
Total size range	0·8—4·4μ	1·4—7·9μ
Size range : pair 1	3·6—4·4μ	4·6—7·9μ
Size range : smallest pair...	0·8—0·9μ	1·4—1·9μ

REFERENCES

BRENNER, S. 'Chromosome studies in *Elephantulus* with special reference to the allocyclic behaviour of the sex chromosomes and the structure of heterochromatin,' M.Sc. Thesis, University of the Witwatersrand. (1947).

DARLINGTON, C. D. 'The internal mechanics of the chromosomes I. The nuclear cycle in *Fritillaria*.' *Proc. Roy. Soc. B.*, 118, 33-59 (1935).

DAVIS, D. H. S. 'The affinities of the South African gerbils of the genus, *Tatera*.' *Proc. Zool. Soc.*, 118, 1002-1018 (1949).

MEASROCH, V. 'Growth and reproduction in the females of two species of gerbil, *Tatera brantsii* (A. Smith) and *Tatera afra* (Grey).' M.Sc. Thesis, University of Cape Town (1948).

ROBERTS, A. 'Report upon a survey of the higher vertebrates of north-eastern Zululand.' *Ann. Transvl. Mus.*, XVIII, 163-251 (1936).

TOBIAS, P. V. 'The characterization of the spermatogonial chromosomes of the albino rat (*Rattus norvegicus albinus*).' *S. Afr. J. Sci.*, XLIII, 312-319 (1947).

Acoustics

A new two-monthly journal devoted entirely to acoustics has appeared. The aims of '*Acustica*' are to help the acoustical scientist to keep abreast of the important developments in his subject and the journal will therefore present the most important results of original research. It is to be international and multilingual though the editors expect that most of the articles will be in English, French or German. Dr. C. W. Kosten of Delft, Holland, is the editor in chief but the three sponsoring organizations, The Acoustical Group of the Physical Society of London, Groupement des Acousticiens de Langue Française and Verbandes der Deutschen Physikalischen Gesellschaften have nominated three of the five editors. The English editor is Dr E. G. Richardson, King's College, Newcastle on Tyne. *Acustica* is published by S. Hirzel Verlag, Zurich.

The following is a summary of some of the articles in the first issues :—

Haas.

Articles based on author's doctorate dissertation on original study of subjective effects of varying time intervals between initial and echo sounds. Subject of fundamental value to study of audibility where sound is amplified and distorted by echoes : for example, in large rooms, telecommunication and public address systems. Subject being developed in America : 'Influence of Interaural Phase on Masking Thresholds. I. The Role of Interaural Time Deviation,' F. A. Webster, *J.Ac.Soc.Am.*, Vol. 23, 1951, p. 452.

Barkechli

As applied to a simple right parallelopiped reverberation chamber, theory is developed, based on well-known reverberation standing wave analysis (P. M. Morse : 'Vibration and Sound,' Chap. 8) to predict variations in reverberation time with position of absorbent panels on chamber walls.' This effect is pronounced below 1,000 c/s as is shown by experimental verification. This analysis would have importance in application to standardization of reverberation chamber procedure.

Aggarwal and Parthasarathy

These Indian authors offer developments of existing theory to explain light diffraction phenomena by ultrasonic waves reported by Bergmann in 1934.

Cremer

Comprehensive review of contemporary knowledge of the mechanism of hearing, stressing the importance of Bekesy's (now at Harvard) experimental findings.

In addition, the Journal contains a list of papers read to the London Physical Society Acoustics Group—Notice of Formation of International Commission on Acoustics—Report on International Auditorium-Acoustics Conference held in Göttingen—Review of a German text book on Acoustics.

Locating Minerals by Plane

Geologists of the United States Geological Survey fly with a magnetometer suspended on a long cable beneath the fuselage. Variations in the earth's magnetism are thus recorded as the plane flies over the prospecting area. Data thus recorded are plotted on aeromagnetic maps which then show variations in total magnetic intensity in particular areas and also indicate differences in composition and depth of bedrock beneath glacial drifts.

Many thousand of square miles have thus been mapped. Such information is open to the public and has proved of great use in locating mineral deposits.