A Chromosomal Survey of Some Indian Insects III. Variations in the Chromosome Number of *Gryllotalpa africana* due to the Inclusion of Supernumerary Chromosomes[1)]

By

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(*With 26 Figures in the Text*)

Introduction

The mole-crickets having the scientific name of *Gryllotalpa*, coming under the order Orthoptera, are quite interesting insects in relation to cytology. vom Rath ('92 '95), Senna ('11), Baumgartner ('11, '12), Payne ('12, '16), Voinov ('12, '14, '25), Winiwarter ('27, '37), Barigozzi ('33) and Steopoe ('39) have made important contributions to our knowledge on the chromosomes found in the germ cells of these insects. On referring to the papers of Voinov ('12, '14, '25), Winiwarter ('27, '37), Barigozzi ('33) and Steopoe ('39), we learn that there occur three distinct local races with differing karyotypes in *Gryllotalpa gryllotalpa*, which are distributed throughout Europe. The material which forms the subject matter of this paper also shows similar karyological variations found in one and the same species. However, the most interesting fact about this Indian material is that these variations in the chromosomal complex are not in any way correlated with localities. The numerical variations in the chromosomal garniture are seen in

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The essential points of the present work have been already reported at the 11th General Meeting of the Genetic Society of Japan at Fukuoka, 1938 (Makino, Niiyama and Asana '38).

a small number of individuals among a group of specimens collected from any one locality. These chromosomal variations are due to the presence of a certain number of supernumerary chromosomes which add to the number of elements in the garniture. In a collection made from any one locality individuals with and without supernumeraries occur together. Numerically, the former are fewer than the latter.

A survey of literature shows that there are a good many instances of variations brought about in the chromosome complex of a species due to the existence of supernumerary chromosomes. The occurrence and significance of supernumerary chromosomes in the Orthoptera too have been recorded in a number of papers. So far as the authors are aware the following investigators have reported upon the numerical variations in the chromosomal complexes of several Orthopterous forms which have resulted from the appearance of supernumeraries in garnitures: Stevens ('12), in *Ceuthophilus,* Carothers ('17) and Helwig ('29) in *Trimerotropis* and *Circotettix,* Robertson ('17) in *Tettigidea,* McClung ('17) in *Hesperotettix,* Carroll ('20) in *Camnula,* Natori ('32) in *Podisma,* Itoh ('34) in *Locusta,* Minouchi ('34) in *Trixalis* and Hareyama ('39) in several species of Locustidae. In this study attention is invited to our assumption that this variability in the chromosomal complex of the Indian forms of *G. africana* does not preclude the possibility that here individuals differing in the chromosomal complex interbreed, that there is free fertilization among them, which may account for the reappearance and perpetuation of the same variability in a certain number of individuals from generation to generation.

The investigation here recorded is not entirely complete owing to the difficulty of collecting additional material. But the information so far obtained we believe to be interesting enough to warrant publication.

We feel greatly indebted to Professor Kan Oguma for his helpful criticism and highly valued advice in carrying out this investigation.

Material and Method

Gryllotalpa africana de Beauvois dealt with in this paper is a common species of Gryllotalpidae (Gryllodea; Orthoptera) with a wide distribution through Asia, Africa, Australia and New Zealand. The specimens on which these observations are based were collected in Western India by one of us (J.J.A.) in the vicinity of the Ismail College, Jogeswari near Bombay from January to March 1934 and from the neighbourhood of the Gujarat College, Ahmedabad, Western India, in the years 1938 and 1939. The identification of the dissected specimens was confirmed by Dr. H. Furukawa of the Tokyo Imperial University to whom our most cordial thanks are due.

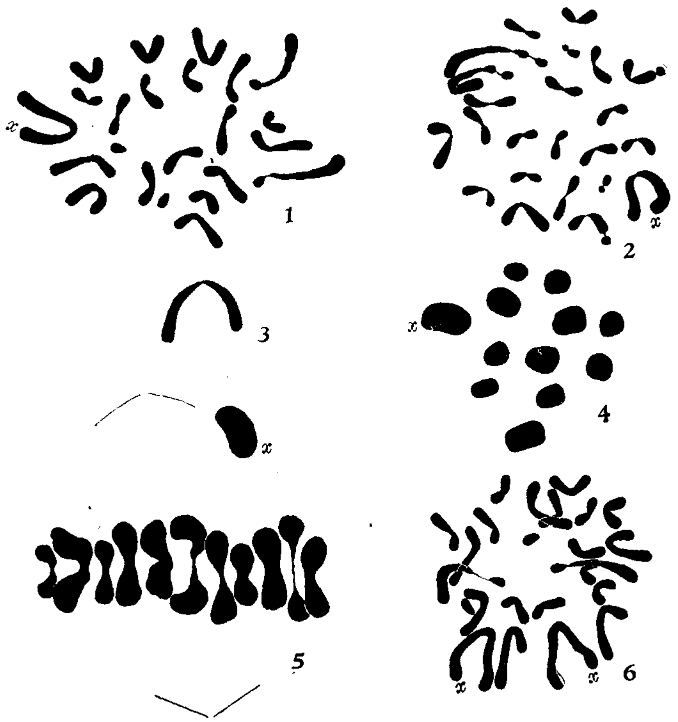
Testes and ovaries were fixed in Benda, in other modifications of Flemming prepared by diluting strong Flemming with varying proportions of distilled water and glacial acetic acid, and in Allen-Bouin. The material thus prepared was subjected to the usual paraffin method, sectioned and stained by the Heidenhain's iron-haematoxylin method.

The camera-lucida drawings reproduced here were made by one of us (S.M.) under a magnification using a Zeiss 1.5 mm. objective and a K 20× ocular.

Observations

The diploid chromosome number, 23 ($2n$), observed in the *Gryllotalpa africana* from India is the same as discovered in the Japanese species examined by Ohmachi ('29, '35). Henceforth in the course of this paper this type, of specimens will be referred to as the 'basic' or 'ordinary' type. Besides this ordinary type other individuals are met with in our collection, which are designated as S^1 and S^2 types according to the number of supernumerary chromosomes, either one or two, which are included in the chromosomal complexes of these two types. Details are given in the following description.

(1) Basic (ordinary) type. Though no difference has been observed, as mentioned above, in the diploid chromosome number between the Indian and Japanese forms (Ohmachi '29, '35), we have to record some difference in the morphology of their individual chromosomes when these two forms are compared. The spermatogonial metaphase of the Japanese form, according to Ohmachi, consists of 5 pairs of rods of varying length, 4 pairs of small V's, a pair of large J's, and a pair of slender, crooked rods, together with an X-chromosome, which is V-shaped. A close examination of the excellent preparations used in this study reveals that this description by Ohmachi is not in accord with our observations and it needs to be somewhat modified. As seen in Figs. 1–2, the chromosomes of *G. africana* all bear distinct constrictions. Since these constrictions are regarded as the attachment loci for the spindle fibres as seen from their behaviour in the process of division, one can justifiably maintain that the chromosome complement of the Indian species consists of all atelomitic elements which vary in size and shape. To make



Figs. 1–6. Chromosomes of the ordinary type. 1–2, Spermatogonial metaphase. 3, X-chromosome. 4, Primary spermatocyte metaphase. 5, Primary spermatocyte meta-anaphase, side view. 6, Chromosomes of the female (ovarian follicle cell). x=X-chromosome. ×3000.

this clear, a serial arrangement of all the chromosomes of the complement has been attempted in Fig. 24, in which supposed pairs of chromosomes have been ranged in order of their magnitude. The first one represents the X-chromosome which is the largest and V-shaped. That the X-element is represented by the largest one of all the elements and that it has a submedian constriction, the arms of the V being not equal[1] will become quite evident by reference to the accompanying figures (*cf.* Figs. 1–3 and 24). The second pair is formed by two prominent elongated elements, each composed of an elongated rod-like part and a globular segment which is connected

1) The X-chromosome described by Ohmachi ('29) has its two arms equal in length.

with the proximal end of the former by a thin thread. In considerably destained slides the rod part of this chromosome shows one more clear constriction near its proximal end, as seen in Fig. 2. Since the latter constriction is nothing more than the attachment locus of the spindle fibre as seen from the behaviour of this chromosome in division, the inference is drawn that the globular element, connected by the thin thread with the rest of the chromosome, is a trabant or satellite, which is often seen in plant chromosomes with a similar structure. The elements from the third to the eleventh (*cf.* Fig. 24) are all V-shaped chromosome pairs most of which have nearly submedian constrictions. Constrictions in these chromosomes are clearly visible if we examine the Fig. 2. It also reveals an additional pair of chromosomes, probably the forth one, where in both the members of the pair one can see a trabant near the distal end of the large arm of V, separated by a clear constriction. The twelfth pair of elements appear as simple rods in ordinary slides. But if they are sufficiently destained, these chromosomes also show prominent constrictions at one of their ends (Fig. 2). From the above evidence one may conclude that the chromosomes of *G. africana* are all atelomitic in structure and that two pairs of complements, the second and probably the fourth, are SAT-chromosomes carrying trabants.

Thus we see that the morphological aspect of the chromosomes of *G. africana* from India differs somewhat from that of the Japanese forms as described by Ohmachi ('29, '35). But we have reasons to doubt whether these differences are real and fundamental, as the Japanese forms of *G. africana* collected in Sapporo and examined by us show a karyotype quite similar to that of the Indian form on which the foregoing description is based. It seems probable that Ohmachi's description was based on deformed chromosome resulting from poor fixation.

In the primary spermatocyte division there occur 12 chromosomes at metaphase, 11 of which are autosomal tetrads, the remaining element being the X-chromosome (Fig. 4). No characteristic structure can be discerned in the autosomal tetrads. In this division the X-chromosome normally runs ahead of the others, without segregation, towards one of the two poles of the spindle (Fig. 6). This mode of division results in two sorts of secondary spermatocytes with respect to the X-element, one including 11 autosomes and the

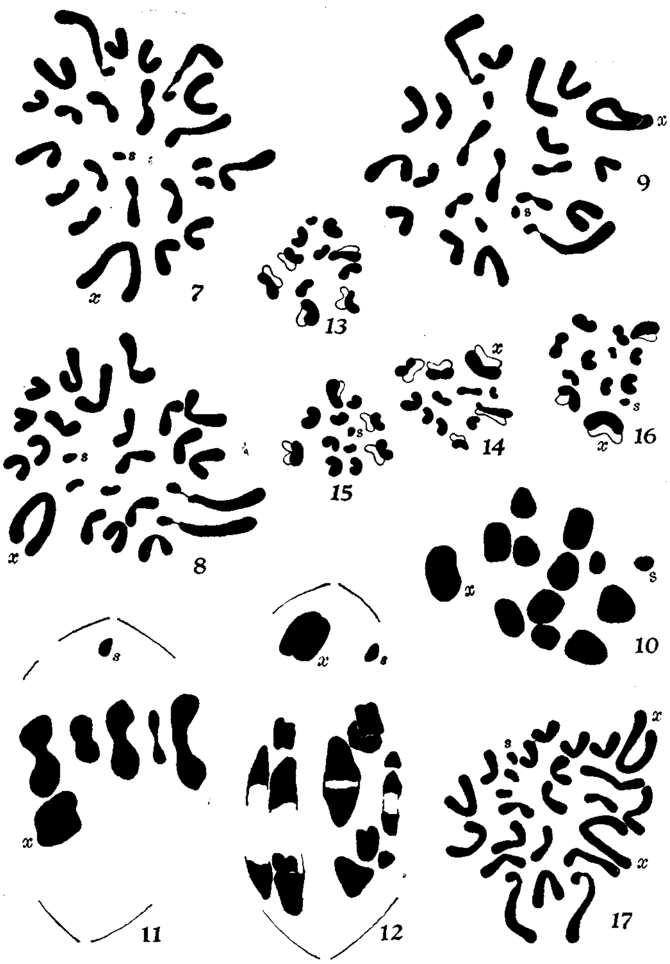
X and the other containing 11 autosomes only. Thus two kinds of sperms, one with and one without the X, there being 11 autosomal elements in both, may originate from these secondary spermatocytes.

The chromosomes of the female were observed in the ovarian follicle cells. The number of chromosomes here is definitely 24 ($2n$) or 22 autosomes and the 2 X-chromosomes. As seen in Fig. 6, all the autosomal elements of the female cell exactly correspond in size and shape to those of the male, excepting the X which is paired here. From this fact it is assumed that every egg cell contains 11 autosomes and one X in its nucleus as a result of maturation.

(2) **S^1 type.** This is the form possessing one supernumerary chromosome in addition to the ordinary complex of chromosomes described above. Accordingly, the diploid number of chromosomes in this type is 24 in the male (Figs. 7–9) and 25 in the female (Fig. 17). It will be observed that other chromosomes of this complex are quite like those of the 'basic' type and do not seem to have undergone any modification due to the presence of this supernumerary element among them (compare Fig. 24 and Fig. 25). The supernumerary, *s*, in these figures is the smallest of all the components of this garniture and is in the form of a minute, grain-like body, about half the size of the 12th chromosome. Fig. 25 shows the size relations of all the components of this complex. In the mitotic division the behaviour of the supernumerary shows nothing abnormal and like other chromosomes of this group it regularly splits into two equal parts.

The primary spermatocyte metaphase of this type shows 13 chromosomes, 11 of which are autosomal tetrads, while there is only one X-chromosome and one supernumerary, the latter two elements being univalent in nature (Fig. 10). In the ensuing division each one of the autosomal tetrads splits into two equal parts, while the two univalents, the X and the supernumerary, do not divide but remain entire and travel to one or the other pole of the spindle independently of each other.[1] Therefore, in some cases, the super-

1) In *Trimerotropis, Circottetix* (Carothers '17), *Metapodius* and *Banasa* (Wilson '07, '09, '10), *Tettigidea* (Robertson '17), and *Podisma* (Natori '32) also the supernumerary does not split in the first division and here its behaviour is quite similar to the present case. In *Locusta* on the other hand, according to Itoh ('34), the disjunction of the supernumerary chromosome at the first division seems to take place commonly and Carroll ('20) has reported that in *Camnula* the supernumerary as a rule, goes entire to one pole in the first division and rarely divides into two parts.



Figs. 7-17. Chromosomes of S1 type. 7-9, Spermatogonial metaphase. 10, Primary spermatocyte metaphase. 11-12, Primary spermatocyte meta-anaphase, side view. 13, Secondary spermatocyte metaphase, no X-class. 14, The same, X-class. 15, The same, no X-class, containing a supernumerary. 16, The same, X-class, containing a supernumerary. 17, Chromosomes of the female (ovarian follicle cell). *x*=X-chromosome. *s*=supernumerary chromosome. ×3000.

numerary and the X-chromosome run together to the same pole (Fig. 12), while in other cases they part company and each reaches the pole opposite the other as seen in Fig. 11. As a consequence of this random distribution of the X-chromosome and the supernumerary in the first division the following four kinds of secondary spermatocytes, as regards the X (x) and the supernumerary (s) are produced: those containing 11 autosomes only (Fig. 13), 11 autosomes with an X (Fig. 14), some with 11 autosomes + s (Fig. 15) and lastly those which contain 11 autosomes + X + s (Fig. 16). In the normal course of events this leads to the formation of four kinds of sperms differing from one another in some of their chromosomal constituents.

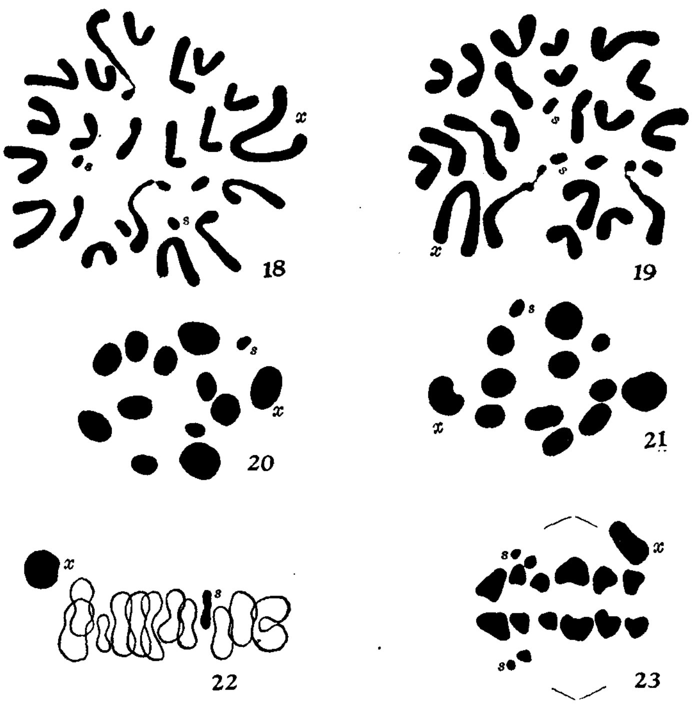
The diploid number of chromosomes of the female in this type as observed in the follicle cells of the ovary is 25, consisting of the autosomal complex of the 'basic' type, a pair of X-chromosomes and a supernumerary. The formula of this type of garniture is 22 autosomes + XX + s (Fig. 17). Assuming that the supernumerary here also does not divide in the first division as was the case with the male, we may expect that egg cells originating from such a mode of division will be of two kinds with respect to their chromosomal organization. They will be of two classes, one being composed of 11 autosomes and the X element, and the other containing 11 autosomes, the X and the supernumerary (s).

It must be emphasised that the occurrence of the supernumerary is not variable but constant wherever it is found, either in mitosis or meiosis of the germ cells of an individual which shows this particular chromosome. Nor does any variation occur in the number of this supernumerary in one and the same individual. The specimen belonging to the S^1 type is definite, constant in its chromosomal organization. In this respect the present case is remarkable as it differs from the Acridian species such as *Camnula* (Carroll '20) and from *Locusta* (Itoh '34) in which the number of supernumerary chromosomes varies concurrently in the same individual.

(3) **S^2 type.** The spermatogonium of this type shows 25 chromosomes at metaphase, consisting of 23 chromosomes, which are in no way different from those which constitute the complex of the 'ordinary' type, and the extra chromosomes (Figs. 18–19). The latter elements are two supernumeraries, which being equal in size and shape constitute a pair of homologous chromosomes. As seen in Fig. 26, these supernumerary elements are distinct bodies, two entire extra chromosomes, and they do not seem to have resulted from any change in the other elements of the garniture. As with

the S[1] type, no chromosomal variation is found within an individual of the S[2] type.

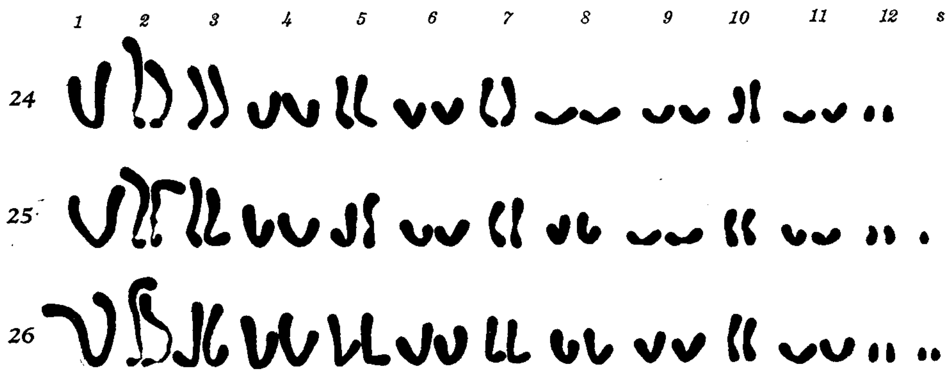
Thirteen chromosomes appear at the metaphase of the primary spermatocyte (Figs. 20–21). They are composed of 12 bivalents and an X univalent. One of these 12 bivalents is formed by the



Figs. 18-23. Chromosomes of S[2] type. 18-19, Spermatogonial metaphase. 20-21, Primary spermatocyte metaphase. 22-23, Primary spermatocyte meta-anaphase, side view. x = X-chromosome. s = supernumerary chromosome. ×3000.

two homologous supernumeraries[1)] of the spermatogonial stage. The smallest among all these chromosomes of the primary spermatocyte metaphase is this extra bivalent which is clearly distinguishable and it always takes a peripheral position (s in Figs. 20–21). The behaviour of the supernumerary bivalent is quite the same as that

1) According to Itoh ('34), in *Locusta* non-disjunction of the homologous supernumeraries occasionally occurs in the first division. In the present material from *G. africana* they always conjugate and form a bivalent.



Figs. 24-26. Paired arrangement of spermatogonial chromosomes in serial order. 24, Ordinary type. 25, S^1 type. 26, S^2 type. *s*=supernumerary chromosome.

of the other ordinary tetrads. In division all the tetrads segregate into two equal halves; so does the supernumerary (Figs. 22–23). Thus, all the elements, including the supernumerary, after segregation get equally distributed to two poles of the spindle, except the X chromosome, which goes undivided to one of the two poles. All the resulting secondary spermatocytes contain one extra chromosome (*s*) besides the autosomal group of the ordinary type, while only one half of these secondary spermatocytes include in their garniture the X, and the other half are without it. Thus one class of the cells contains 11 autosomes + s + X, and the other has 11 autosomes + s only (*cf.* Figs. 15 and 16.).

In the material so far examined, we regret, no female belonging to the S^2 type possessing 2 supernumeraries, has been discovered. But this is not surprising in view of the fact that these two aberrant types, S^1 and S^2, are comparatively very few and occur in such a low percentage as seen in Table I. Moreover, this study has not been based on an examination of a large number of specimens. However, the occurrence of the S^2 female in nature may not be unreasonably inferred from a consideration of the chromosomal relationship existing among these three types, if we assume that they interbreed freely. An attempt has been made, as stated below, to show that a female containing eggs with the chromosomal organization, 11 autosomes + X + s, may not be so infrequently produced in nature if free fertilization prevails among these three types.

A discussion on the chromosomal relationship of the three types

As mentioned above, the Indian forms of *G. africana* fall into three distinct categories, though not equally numerous, in respect of the chromosomal organization of their germ cells. Each karyotype is distinctive, is confined to the individual exhibiting it and shows no variation in that individual. In the specimens which we have so far examined the frequency of occurrence of these three types is given in Table 1.

TABLE I

Type	Ordinary type	S^1 type	S^2 type	Total
Number of specimens	42	4	3	49
%	86 %	8 %	6 %	100 %

It is highly probable that in these Indian forms of *G. africana*, which show their internal differentiation in their chromosomal organization but inhabit the same locality, crossing occurs freely. If we briefly summarize our foregoing observations as shown in Table II, it will be seen that 4 kinds of sperms, 11a, 11a + X, 11a + s, 11a + X + s, and 2 kinds of eggs 11a + X and 11a + X + s, are produced by these three types, namely the 'ordinary', the S^1 and S^2 types.

TABLE II

Male cell			Female cell		
Type	Spermatogonium	Sperm	Type	Oogonium	Egg
Ord. type	23=22a+X	11a 11a+X	Ord. type	24=22a+XX	11a+X
S^1 type	24=22a+X+s	11a 11a+s 11a+X 11a+X+s	S^1 type	25=22a+XX+s	11a+X 11a+X+s
S^2 type	25=22a+X+2s	11a+s 11a+X+s	S^2 type	26=22a+XX+2s	11a+X+s

a: autosomes. s: supernumerary. X: X-chromosome.

If we assume, as indicated in the diagram below, that crossing between these four kinds of sperms and two kinds of eggs occurs freely in nature, then their reappearance and perpetuation in the three types is established. This may explain why these three different karyotypes, the 'ordinary', the S^1 and S^2, remain unaffected and that

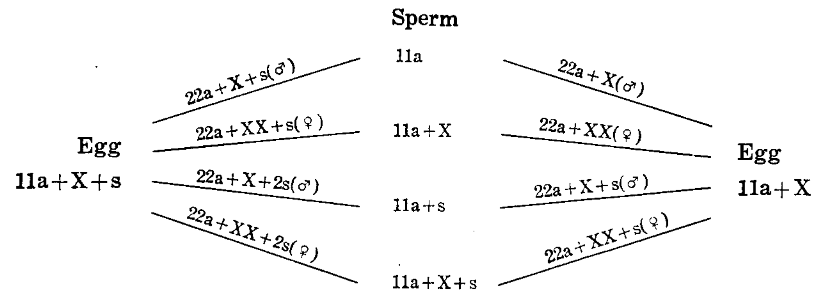


Diagram showing the combination of chromosomes in fertilization

there is neither progressive increase nor decrease in the number of supernumeraries from generation to generation.

As regards the origin of the supernumerary chromosomes in this species we are not in a position to make any statement. The explanation that they may arise as a result of the fragmentation of the other chromosomes of the garniture is in a large measure discounted by the observation already made that no change in the size or morphology of these other chromosomes is noticeable. Irrespective of their origin, however, it is interesting to note that the appearance of the supernumeraries does not seem to effect any visible change in the character of the phenotype of the animal. This seems to indicate that supernumeraries are probably geneless or inert.

Closely related to the condition of the supernumeraries seen in *G. africana* are the observations made by Minouchi ('35) on the germ cells of the beetle, *Zabrotes subfasciatus*. According to him, in *Zabrotes* a supernumerary element is always found in an unpaired state either in the male or the female cell. In the male, the supernumerary passes to one pole without segregation in the primary spermatocyte division. Consequently, there arise 4 kinds of sperms with respect to the X and the supernumerary. In the female, the situation is different. During the first maturation division of the ovum, the supernumerary is cast off outside the nucleus. It has thus no chance to enter the ripe egg nucleus. On this account, in *Zabrotes* the supernumerary is always transmitted in an unpaired state from generation to generation. In this respect, therefore, *Zabrotes* is somewhat different from the present case.

Quite recently Steopoe ('39) has published his observations on *Gryllotalpa vulgaris* collected in Roumania. The majority of specimens had in their germ cells 14 chromosomes, including the X and the Y, as the diploid number. But in certain individuals a few cells were discovered which contained 15 chromosomes due to the presence of an additional minute element almost as large as the Y. The origin and behaviour of this extra chromosome have been questioned by the author. It seems to us that the latter element may perhaps be of the same nature as the supernumerary here described as occurring in some Indian forms of *G. africana*.

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