High Chromosome Number and Giant Postreductional Sex Chromosomes in the Beetle *Walterianella venusta* Schaufuss (Chrysomelidae, Alticinae)

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INTRODUCTION

In the beetles, nine pairs of autosomes and the sex chromosomes X and y (y smaller than X) are so common that 9+Xy is regarded as the basic formula for the Coleoptera, or, at least, for Polyphaga. Male is the heterogametic sex. In the spermatogenesis of most polyphagous beetles, X and y pair to form a parachutelike bivalent (Xy_p), which is prereductional. The mode of pairing is not completely understood. Either chiasmata (*11*)[2] or nucleolus (*4*), or both, are responsible for it.

Among the over 700 species of beetles so far checked cytologically numerous exceptions occur from the main formula $9+Xy_p$ (*12,13*). The lowest number, accompanied by a neo-Xy formation, is encountered in certain elaterids (*9,20*), whereas the highest numbers occur, if parthenogenetic forms are not taken into account, in some chrysomelids (*2,22,23*) and curculionids (*16,17,18*). The total width of variation is from 4+neo-Xy to 28+XXy. Both extremes show, probably not completely incidentally, an exceptional sex-chromosome mechanism. The sex chromosomes form a susceptible focus for initiation of evolutionary changes in the chromosome complement of the beetles. All changes tend to revert to the Xy condition, however.

In the Chrysomelidae, a family with considerable variation of chromosome relations, certain representatives of the subfamily Alticinae, also considered as a separate family Alticidae by some taxonomists, show very unusual chromosome complements and sex-chromosome behavior. The autosomes form 10 (*Altica chalybea, 15*; *Alagoasa* sp., *18*), or 11 (*Altica* ssp., *12,13*) bivalents in the spermatogenesis, the sex chromosomes pairing very late, or not pairing, but coorientating just prior to the anaphase I. Numerous irregularities in the behavior of the sex chromosomes, as well as their different sizes and shapes in related species, suggest a recent formation not well established in all cases.

The present paper deals again with another different case of Alticinae. It is to be hoped that the following observations, although based on one

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[2] Italic numbers in parentheses refer to Literature Cited, p. 163.

single species, will promote the understanding of the chromosomal evolution in this cytologically interesting group. Maybe they will also assist investigators looking for new, unusual objects for use in genetic and radiation experiments.

MATERIAL AND METHODS

Ten males of *Walterianella venusta* Schaufuss were collected at the Instituto Tropical de Investigaciones Científicas of the Universidad de El Salvador, Central America[3]. The species is attracted by light, and is very common in the rooms and on mosquito screens at night. Material was made up into permanent squash preparations according to Smith (*10*). The stain was leucobasic fuchsin, or acetocarmine. The Ortholux microscope and Makam camera, both made by Leitz, were used for observations and pictures. The negative material used was Kodak Ortho Contrast Process film.

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OBSERVATIONS

The earliest stages where the chromosomes could be checked were some spermatogonial metaphases. It was seen immediately that a chromosome complement comparable with that of *Alagoasa* was present. Although the exact number of the dotlike chromosomes could not be determined, they were clearly more numerous than in *Alagoasa*, at least 34. In addition, there were two huge metacentric chromosomes, of which one was nearly mediocentric and longer than the other. Undoubtedly these big, heteromorphic chromosomes are the sex chromosomes. Hereafter they are called "X" (the longer one), and "y" herein. It is not usual to see untreated insect chromosomes in metaphase, with the chromatids far apart from one another, but these giant sex chromosomes show that phenomenon in most spermatogonial metaphases encountered. Some of the clearest preparations support Lima-de-Faria's (*5*) opinion that the last contact is formed not by the centromere itself but by the adjacent short segments (fig. 1).

The contents of a young, growing spermatocyte I consist of a bouquette-like-arranged chromatin. There is a heterochromatic body at the "bottom", and two arches of euchromatin opposite it, and about 20 or 30 dots, most of which represent small individual bivalents. It is hard to follow the confluence of these structures at the earlier stages. Later on, it becomes obvious that the basal heterochromite was formed by one arm of the sex chromosomes (fig. 2,A,B). The attraction between the heterochromatic arms

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seems to bring the sex chromosomes together, whereas their euchromatic parts are indifferent.

In diakinesis, where the polarization has disappeared, the sex chromosomes move apart from one another (fig. 2,C,D). There is still a noticeable difference between the condensation of the arms in both X and y.

The sex chromosomes remain separated in metaphase I, but are brought to the plate. Their arms are clearly double, the short arms being still slightly differentially condensed. The number of the autosome bivalents is 22 (fig. 3). Curiously, these latter are quadripartite formations like two dumbbells together parallel. In anaphase I all autosome bivalents move apart to 22 chromosomes, and the sex chromosomes divide equationally (fig. 4,A). X and y show a strong condensation at this stage.

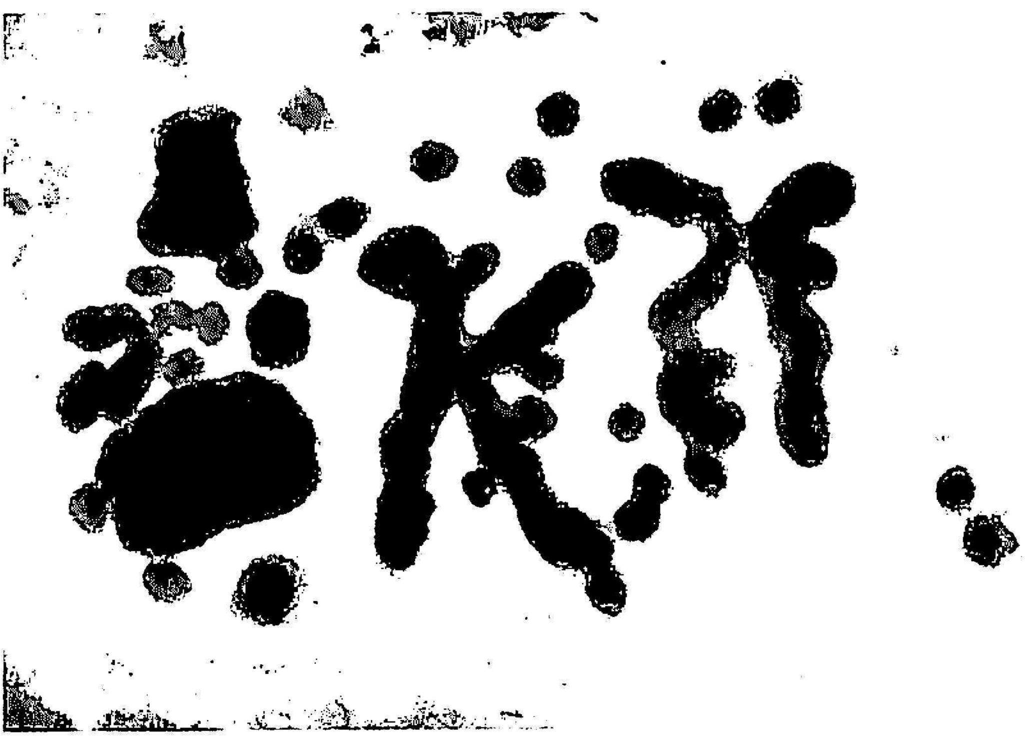

FIG. 1.—Spermatogonial metaphase: Over 30 dotlike autosomes and the giant sex chromosomes, their chromatids wide apart from one another. Note that in y (at the right), the last contact is at both sides of the centromere. Stain: Acetocarmine. Magnification 3600×.

After the interkinesis, in prophase II, the chromosomes appear to be smooth like the socalled "lamp-brush chromosomes." This was the only stage where the short autosomes could be seen to be metacentrics (fig. 4,B). In the following metaphase II all chromosomes are more condensed. The sex chromosomes are found together, the euchromatic arms usually lying parallel (fig. 4,C). A few cases of anaphase II were seen, each anaphase group having only one sex chromosome, either X or y.

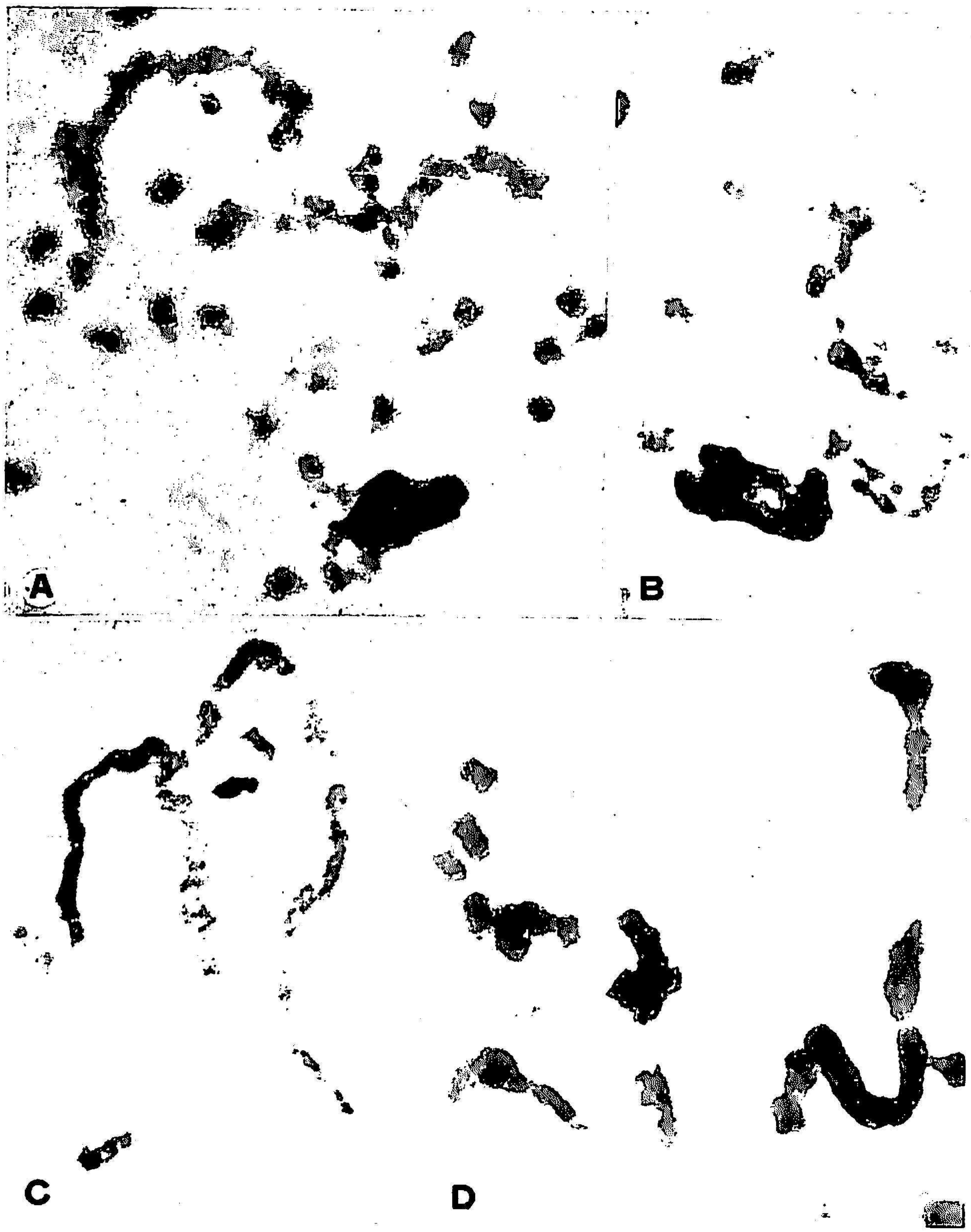

FIG. 2.—A, Diplotene; heterochromatic segments of X and y paired, euchromatic segments unpaired and opposite the heterochromatic body. Most of the short euchromatic bodies are autosome bivalents, but some of them belong to the sex chromosomes. B, later diplotene; the continuity of the euchromatic segments with the heterochromatic ones becomes more obvious. Loose pairing of the euchromatic segments. C, Late diplotene; desynapsis of the sex chromosomes. D, Diakinesis; the sex chromosomes separated, their longitudinal structure still well visible. Stains: Acetocarmine in B, leucobasic fuchsine in others. Magnification 2665×.

According to these observations, the chromosome formula of *Walterianella venusta* is 22+X+y, the sex chromosomes being desynaptic and postreductional.

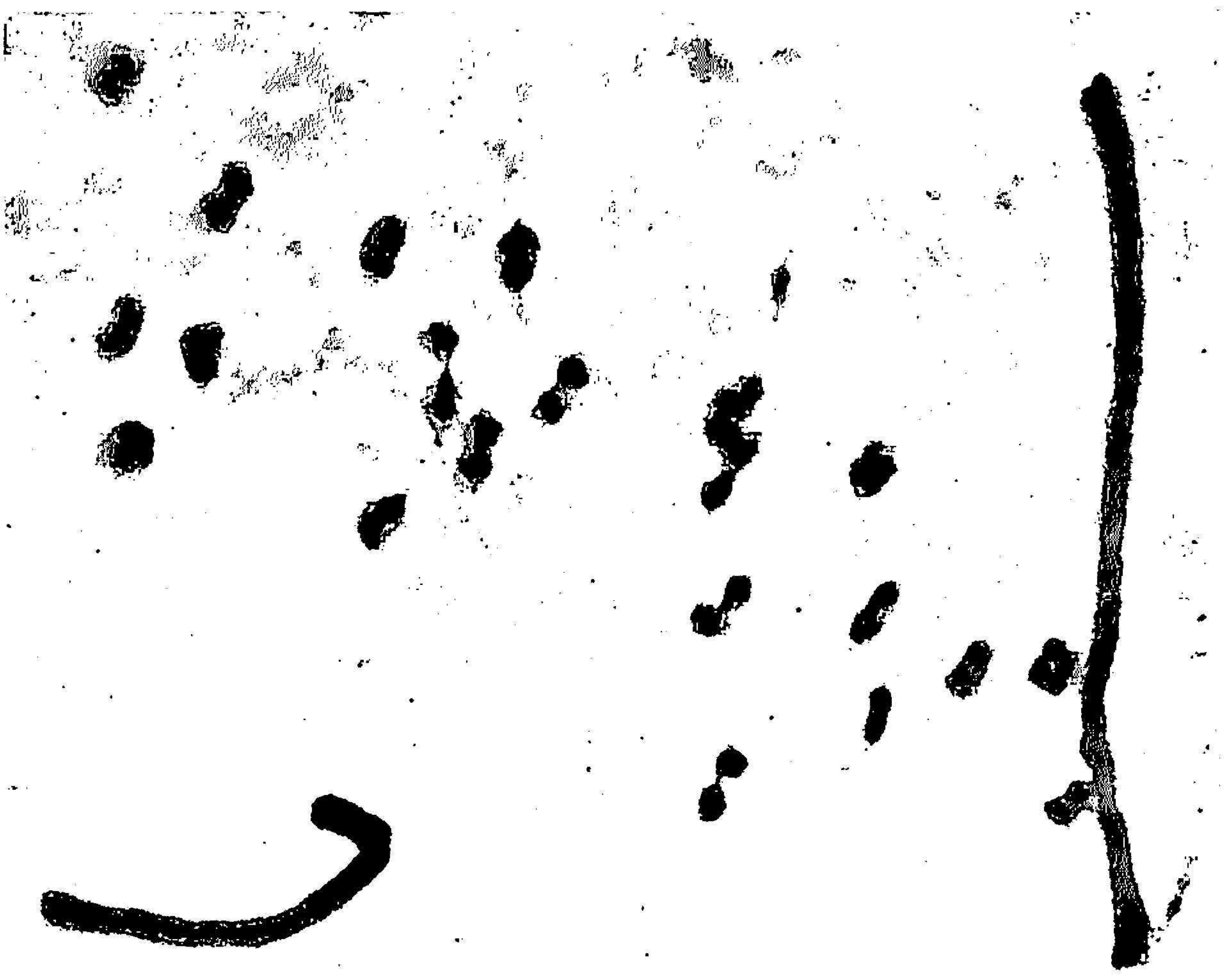

FIG. 3.—Metaphase I, 22 + X + y. Note the double chromatids in the sex chromosomes as well as in the bivalent autosomes. Stain: Leucobasic fuchsine. Magnification 2340X.

DISCUSSION

The variation of the chromosome relations in the subfamily Alticinae is quite wide. There are species like *Blepharida rhois* Forst, wherein the Xy_p structure of the sex bivalent, typical for most Coleoptera, is preserved (*13,14*), or like *Syphraea quintanillai* Bechyné, which has the Coleopteran-typical nine autosome pairs, but the X chromosome only in the male (*21*). On the other hand, there is a large sector where the chromosome complement is characterized by oddly behaving sex chromosomes, and smaller autosomes. The number of autosomes is increased, most *Altica* species having 11 pairs, and the *Alagoasa* species 10 pairs. In *Disonycha*, the relations are most variable. In a small sample of *Disonycha* from Central America, the writer determined the following formulas: 15+X+X, 17+X+X, 18+X+X (*21*). *Disonycha alternata* Ill. from Ontario has 24+neo-XY, according to Manna (in *13*).

FIG. 4.—A, Anaphase I, equational division of sex chromosomes. B, Prometaphase II; 20-22 metacentric autosomes visible, and X and y, all smooth-appearing like lampbrush chromosomes. C, Metaphase II; X and y paired, some autosomes visible. Stains: Leucobasic fuchsine in B, acetocarmine in others. Magnification 3240X.

The systematical limits of the occurrence of this type of chromosome relations have not been worked out at the present. However, the genera wherein they do occur, including *Walterianella*, are systematically close to one another. This suggests a monophyletic evolution, especially because the rearrangements occurring are not quite the usual ones. These chromosome relations look recent, and undoubtedly still frequently produce new forms. A big difference in the chromosome complements of closely related species is a sign of this, as well as the frequent disturbances in the sex chromosome behavior. *Disonycha* probably is close to the main center of the development.

Considering the evolution of the chromosomes in these alticines, one matter can be accepted as a postulate: The high numbers must be derivatives of lower, more Coleopteran-typical numbers. There remain three particular questions of high interest: 1, How are the big sex chromosomes formed? 2, How does the autosome number increase? 3, How did the sex chromosomes of *Walterianella venusta* turn from prereduction to postreduction?

As mentioned above, there is one observation of neo-XY formation in *Disonycha*. In *Alagoasa* no sign of recent rearrangements were seen in the sex chromosomes, but in *Walterianella venusta* the prophasic polarization and structure of both of the sex chromosomes suggests that they have one arm of autosomal, and the other of old sex-chromosomal origin. At first it seems that the autosome on which the sex chromosome has been translocated was of considerable size. The lack of pairing of these arms in the early prophase of spermatocyte I indicates that they are not homologous. They are seen paired only in metaphase II, and it is quite questionable whether that pairing is of any specific nature.

If a nonspecific attraction is responsible for the segregation of sex chromosomes, it is possible that they can both collect and maintain different mutations and rearrangements. So far we know of no noticeably long alticinean autosome which could have served as basis for this neo-XY formation. Probably the euchromatic arms of X and y have received material from different autosomes. In diplotene the arms show various short blocks which cannot be separated by sight from the autosomes. Anyway, it seems probable that neo-XY formation is occurring here, perhaps repeatedly, adding more and more to the size of the sex chromosomes.

According to illustrations by Stevens (*15*) there is little difference between the size of autosomes and sex chromosomes of *Altica chalybea*, the former being bigger, the latter much smaller than in *Alagoasa* and *Walterianella*. *Altica chalybea* apparently represents more conventional alticinean karyotypes, from which *Alagoasa*- and *Walterianella*-type differentiation started. Whether other events like duplications or linear growth, as in dipteran giant chromosomes, are involved in formation of the big sex chromosomes of *Alagoasa*, and *Walterianella*, it is impossible to say. Because we do not understand the development of these sex chromosomes with certainty, and because their euchromatic arms seem to be inhomologous, it is preferable to call them X and y rather than neo-X and neo-y.

Provided there were a drift of autosomal arm-material being repeatedly translocated on sex chromosomes, the final result, when a certain safe minimum of autosome length had been attained, would be like the chromosome complement of *Alagoasa*. This would maintain the original autosome number, but would not increase it. Thus there must be other methods of increasing it.

If chromosomes have been deprived of their major genes, either by recent gross rearrangements or by a long course of evolution, they may accumulate in chromosome complements without too powerful genetic effects, as we know in the case of accessory or supernumerary chromosomes. However, the small autosomes of the alticines are not supernumerary chromosomes. So far their number seems constant for a species. No meiotic mis-segregation has been noted, although it cannot be excluded as a possible means of increasing the chromosome number. The possibility of multiplication of the autosome number by fragmentation does not seem feasible, because they are provided with localized centromeres, and are metacentric in both *Alagoasa* and *Walterianella*. There remains one very hypothetical but intriguing possibility: could it be that the small autosomes are doubled by endomitosis, the cyclically differently-behaving sex chromosomes remaining single? The most direct observation suggesting such a possibility is, that in *Walterianella venusta*, the size of autosomes is similar, but their number is almost double as compared with *Alagoasa*, and exactly double as compared with most *Altica*.

Furthermore, the sex chromosomes of *Walterianella venusta* are very precocious in the separation of chromatids. The chromatids are already apart from one another in the premeiotic mitoses, as in colchicine mitoses. In the spermatocytes they are so advanced in their reduplication-division cycle, as compared with the onset of polarity, that they enter the prophase in a state where chiasma formation is no more possible, and in the first metaphase, their centromeres are prepared for autoorientation, and for equational division.

But the autosomes have also almost divided in the first metaphase. If they were still more precocious in relation to the spindle development, their centromeres would probably be able to autoorientate, and the bivalents would divide postreductionally.

Besides the common cyclic discrepancy between sex chromosomes and autosomes in the spermatogenesis, there are numerous cases where a part of the chromosome complement has a different cyclic behavior than the rest of chromosomes (*1,3,6,7*). Metz (*8*) reported somatic reduction, and duplication of the haploid sets thereafter in a mosaic salivary gland of *Sciara*. A partial reduplication of the chromosome set inside the nucleus has apparently never been observed, but the possibility is not excluded.

Perhaps it could occur in *Walterianella*, if either the sex chromosomes or the autosomes were completely doubled before the division began, and the individualized chromatids divided like chromosomes in the next mitosis.

Provided all genes associated with the sex determination have been transferred to the big sex chromosomes, the doubling of the latter would result in sex disturbances in generations following the doubling, but the doubling of the small autosomes would not. Even formation of multivalents would be prevented by the small size and low chiasma frequency in these chromosomes.

Simplifying the above, the following working hypothesis is put forward: Two drifts operate in the evolution of the alticine chromosomes, first, a trend toward autosomal chromatin being incorporated in the sex chromosomes; second, multiplication of the small autosomes.

We have already answered the third question: The sex chromosomes turned to postreduction because their centromeres were capable of autoorientation in the first meiotic metaphase. In light of the alticinean cytology it seems that a system wherein the autosomes are prereductional, and the sex chromosomes postreductional, arises through a cyclic discrepancy between autosomes and sex chromosomes, or, better, between the onset of the polarity and the division-ability of the sex chromosomes. When the course of development is underway varying irregularities in behavior of the sex chromosomes are seen during meiosis (*Alagoasa*). An intermediate system wherein prereductional segregation of X and y is secured by a sort of short "residual attraction" in metaphase I, is still quite stable and has survived in numerous alticine species. A new stability is attained when the division-ability of the autosome bivalents (cooriented centromeres) and sex univalents (autooriented centromeres) coincide well with the fully formed metaphase spindle, as in *Walterianella venusta.*

SUMMARY

A high chromosome number for a coleopteran, 22+X+y, was encountered in an alticine, *Walterianella venusta* Schaufuss. The autosomes are very short, metacentric; the sex chromosomes very long and also metacentric. The sex chromosomes are desynaptic and postreductional in spermatogenesis.

It is assumed that two trends operate in the evolution of the alticine chromosomes, first, the autosomal chromatin being incorporated repeatedly in the sex chromosomes, and second, the occurrence of the multiplication of the short autosomes.

RESUMEN

Se encontró un número alto de cromosomas (22+X+y) para un coleóptero; esto fue en un alticino, *Walterianella venusta* Schaufuss. Los autosomas son muy cortos y metacéntricos y los cromosomas del sexo muy largos y también metacéntricos. En la espermatogénesis, los cromosomas del sexo son desinápticos y postreduccionales.

Se supone que en la evolución de los cromosomas de los alticinos operan

dos tendencias; primeramente, la cromatina autosomal se incorpora repetidamente en los cromosomas del sexo, luego, los autosomas cortos se multiplican.

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