CYTOGENETICS OF TRIATOMINAE. III: A STUDY ON MALE STERILITY INDUCED THROUGH HYBRIDIZATION OF TRIATOMA SORDIDA AND TRIATOMA PSEUDOMACULATA

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Males from bilateral crosses between Triatoma sordida *and* Triatoma pseudomaculata *were unable to give offspring, as shown by subsequent backcrosses (BC) between hybrid males and parental females. This kind of sterility induced through interspecific hybridization seems to be due to lack of sperm migration from the bursa copulatrix to the spermateca, thus suggesting primarily failure on the part of hybrid males to produce and/or to incorporate male accessory secretions into the spermatophore bulb. Addicional proof that sterility induced in hybrid males is at the sperm level has been afforded by the spermatogenesis herein studied. The anomalous processes like; 1) prophases of spermatogonia with the chromosomes scattered in the cytoplasm, 2) first metaphases with unpaired tetrades, 3) spermatids differing in size and 4) spermatozoa of abnormal shape and generally of giant size, can be taken as an indicator of the degree of departure from the normal course of spermatogenesis.*

INTRODUCTION

Perlowagora-Szumlewicz [7] has reported male sterility induced through interspecific hybridization of Triatominae. Males from unilateral and reciprocal crosses were unable to give offspring, as shown by backcrosses between hybrid males and parental females, while hybrid females backcrossed to parental males proved fertile; eggs developed into vigorous adults. Furthermore, adult progeny from the subsequent backcrosses behaved like the progeny from the first backcross, that is to say, that males were again unable to engender offspring from parental females. The Author suggested that the primary component of the induced sterility was a failure on the part of the hybrid male to transfer sperm successfuly during mating. Holt & North [5] in studies conducted with cabbage loopers had encountered similar problems with the transmission of sperm by irradiated males.

Evidence presented by Dawey [1] demonstrated that in *Rhodnius prolixus*, like in many other insects [2, 4, 5], migration of spermatozoa to the spermatecae is a result of contraction in the female ducts set up by a milky granular secretion, originated in one of the four lobes present in the

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accessory glands of the male. This leads to a possible speculation on the inability of hybrid males to produce and/or to incorporate the milky seminal fluid into the spermatophore bulb.

There is evidently a need to have more information on the sterility induced through interspecific hybridization. It would be necessary, for instance, to circumvent the migration barriers through surgical manipulations in order to know whether the sperm is functional, experiments as yet not done. However, indirect information by Schreiber et al. [9, 10] revealed anomalous spermatogenesis in hybrids from an unilateral cross between *T. infestans* and *T. pseudomaculata*. Our objectives were therefore to determine whether similar phenomena occur in hybrids from bilateral crosses between *T. sordida* and *T. pseudomaculata* and in those obtained from backcrosses to parental members.

MATERIAL AND METHODS

Hybrids used in the test eexperiment were developed in the Laboratory of Biology and Control of Chagas' disease Vectors, Jacarepaguá, Rio de Janeiro, RJ., where colonies of parent insects have been maintained since 1969. *T. sordida* originated from the State of São Paulo, where it was, at the time of collections, peridomestic, confined mainly to primitively constructed chicken houses. *T. pseudomaculata* has been brought in from the state of Pernambuco where it is mainly peridomestic.

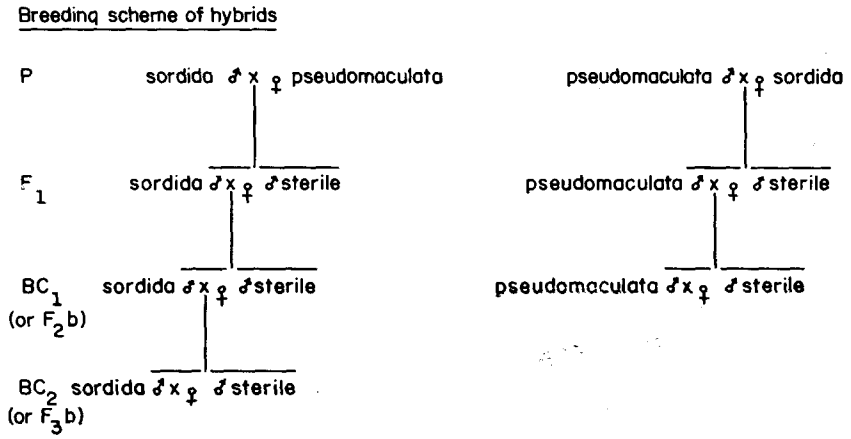
Male and female 4th instar nymphs, distinguished by the pattern of the 9th abdominal sternite [6], were kept separately until they developed into adults. Single pair matings have been performed by placing one virgin female of one species with a virgin male of the other species. In backcrosses hybrid females were exposed to parental males as shown in the breeding scheme.

Ethanol was used to fix the testicles. The lactic orcein squash procedure was followed in the study of spermatogenesis. Spermatids which resulted from two meiotic divisions with no further divison were our choice for the caryometric study of nuclei variability in drawings done with a Camera lucida at 1400 diameters. For comparative purpose histograms of nuclei in parent and hybrid males were made.

RESULTS

Chromossomes scattered in the cytoplasm have been detected in both gonial and meiotic prophases. The most significant alterations observed in hybrids consisted of nonpairing of chromatids in the 1st meiotic metaphase, as shown in Fig. 4, 5, 6, 7. There are seen frequently groups of unpaired chromatids and in a lower than diploid number. The spermatogonial prophases are disrupted and the chromosomes scattered in the cytoplasm.

Histograms showing nuclear diameters of the spermatids of parent species, as seen in Fig. 19, are regular and unimodal. The size of spermatid-nuclei, however, varies with the species. Depending on the paternal species involved ,histograms of nuclear diameters of spermatids in hybrid males also varies. Thus, histograms for male progeny,



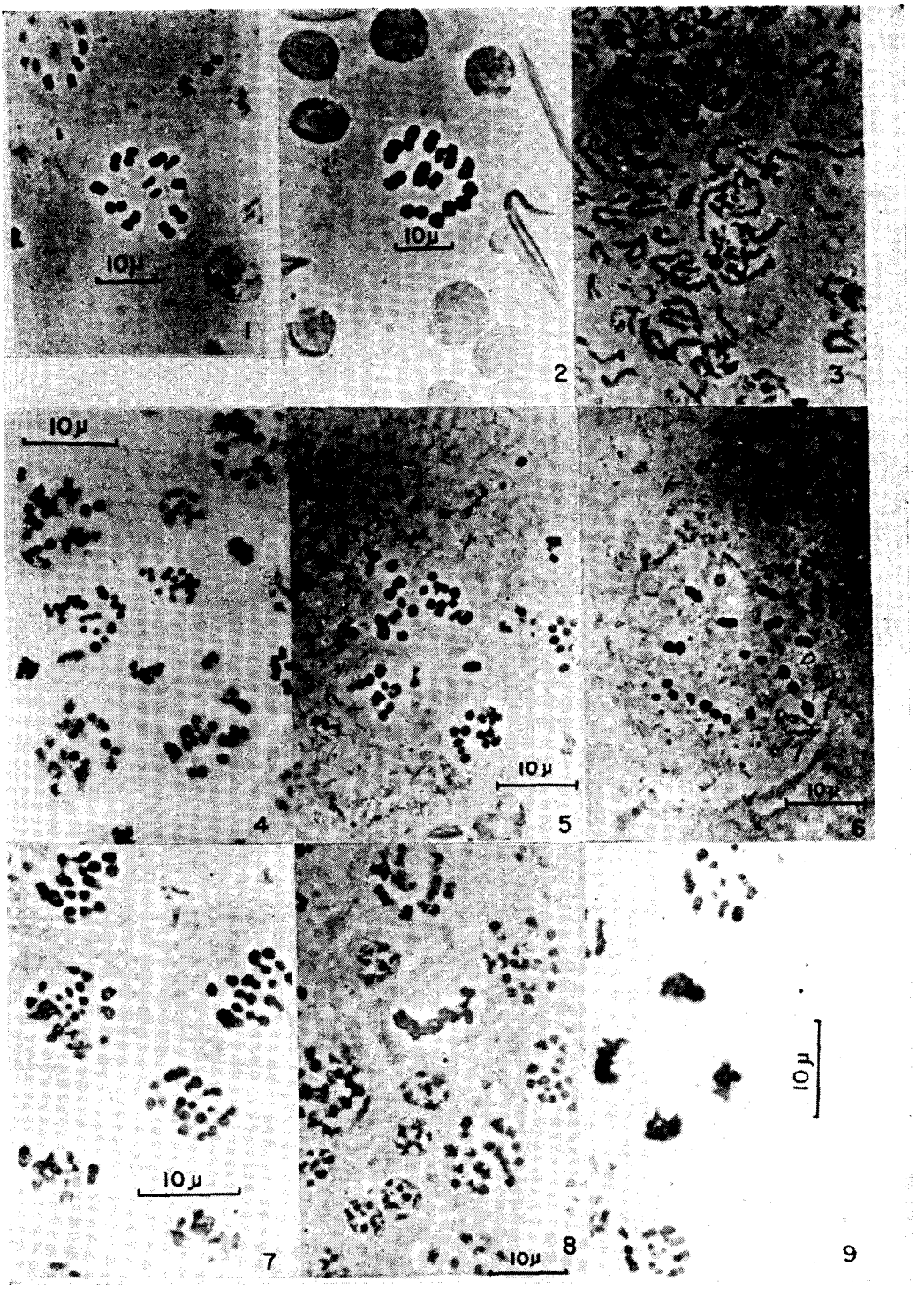


Fig. 1-9: 1. — 1º metaphase in *Triatoma pseudomaculata*; 2. — 1º metaphase in *T. sordida*; 3. — scattered chromosomes of a broken prophase; 4, 5, 6, 7. — 1º metaphase with unpaired chromosomes in F_1 hybrid; 8. — 1º metaphase with fused chromosomes in F_1 hybrid; 9 — 1º anaphase tetrapolar in F_3b hybrid.

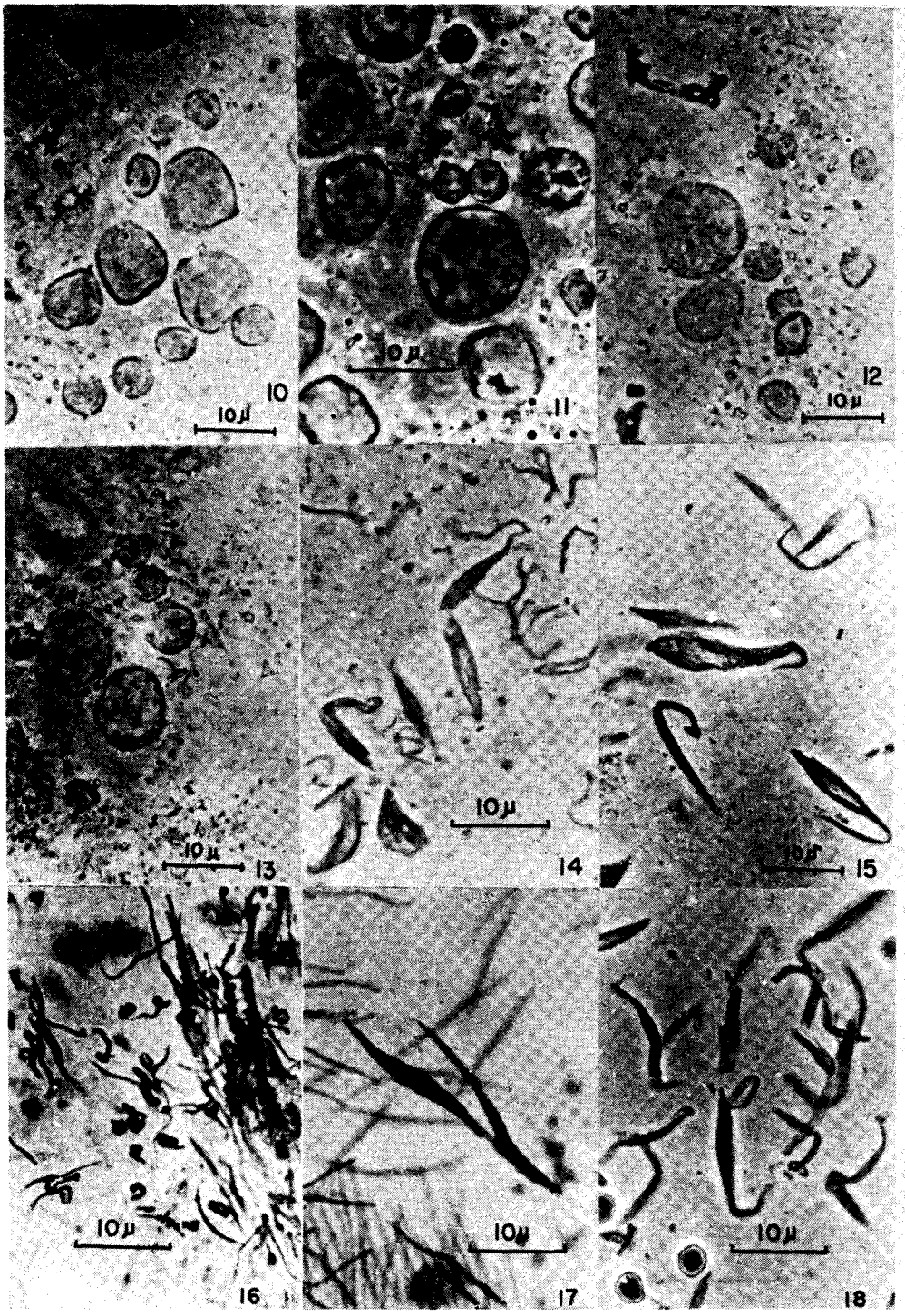


Fig. 10-18: 10, 11, 12. — spermatids of various sizes in F_1 hybrid (*pseudomaculata* ♀ x ♂ *sordida*); 12. — anaphase with fused chromosomes; 13. — spermatids of various sizes in F_2b hybrids; 14, 15. — abnormal and giant spermatozoa in F_3b hybrids; 16. — abnormal spermatozoa in F_1 hybrid (*pseudomaculata* ♀ x ♂ *sordida*); 17. — giant spermatozoa in F_1 hybrid (*sordida* ♀ x ♂ *pseudomaculata*); 18. — giant and irregular spermatozoa in F_3b hybrid (F_2b ♀ x ♂ *sordida*).

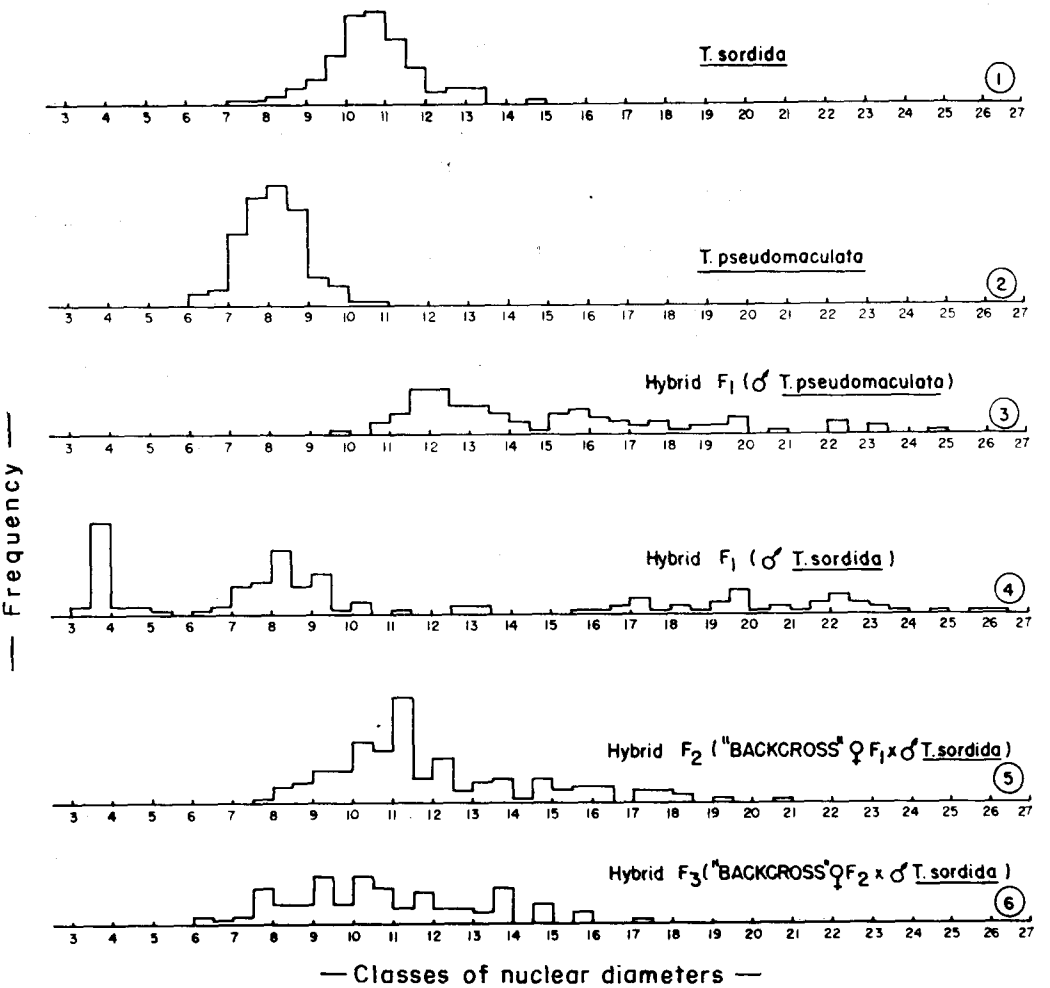


Fig. 19 — Frequency distribution of nuclear diameters in spermatids of hybrids and parent-species.

derived from crosses between *sordida* male and female *pseudomaculata* and from backcrosses to *sordida* male, differ from histograms for male progeny originated in the reciprocal crosses, that is to say, from crosses between *pseudomaculata* male and *sordida* female. Histograms of nuclear diameters of spermatids in hybrids from two subsequent backcrosses to *sordida* male show large variation in size but no definite modal values. It seems noteworthy that the histograms of nuclear diameters of spermatids in F_1 hybrids from the same cross shows modal values higher than haploid and also remarkable modal values lower in size than haploid. A tentative explanation of that may be given by the 1st metaphases with a great dispersion of groups of unpaired chromatids and groups that form isolated nuclei at the spermatid stage, as seen in Fig. 5, 6. The mechanism responsible for the fusion of groups of chromatids is also involved in the formation of spermatids with more than diploid or tetraploid numbers, as seen in Fig. 10, 11, 12, 13.

The spermatids described above form spermatozoa of abnormal morphology and giant size, as seen in Fig. 14, 15, 17, 18.

Bundles of apparently normal spermatozoa can still be seen in hybrids from the 1st and 2nd backcross, but never in those from the 3rd backcross. The latter demonstrate an extraordinary variation of abnormal spermatozoa. The group of bizarre spermatozoa seen in Fig. 16 is not specific for hybrid males.

CONCLUSIONS AND COMMENTS

Schreiber et al. (11) in cytogenetic studies conducted with irradiated Triatominae and with bugs exposed to insecticides (unpublished experiments) had encountered anomalous stages of spermatogenesis similar to those in hybrids herein reported. Therefore it is presumed to be a general reaction caused by any condition disturbing meiotic processes.

It has been also reported by Schreiber & Pellegrino (8) that blocking of the metaphase with colchicine and subsequent reconstitution of the nuclei gave cells similar to spermatids herein described; some of normal appearence with *n* chromosomes, others with *2n* and *4n* chromosomes. It is tentatively concluded that the spermatozoa produced by these spermatids, abnormal in shape and size, are not functional. Therefore, it is concluded that the sterility induced in Triatominae through interspecific hybridization does occur at the sperm level.

RESUMO

Os machos provenientes de cruzamentos bilaterais entre Triatoma sordida *e* Triatoma pseudomaculata *revelaram a incapacidade de produzir progênie, como se demonstra nos retrocruzamentos de machos híbridos com fêmeas das espécies paternas. Esta modalidade de esterilidade induzida através da hibridação interespecífica, parece ser devida à ausência de migração do esperma, determinada pela incapacidade dos híbridos machos produzirem a secreção opaca em suas glândulas acessórias e/ou incorporá-la à luz do espermatóforo. A prova adicional de que a incompatibilidade induzida em triatomíneos, através da hibridação interespecífica, está no nível do esperma é fornecida pelo tipo anômalo de espermatogênese em híbridos aqui descrito, mostrando deste modo que: as prófases espermatogoniais têm os núcleos disruptos e os cromossomos espelhados no citoplasma. As primeiras metáfases meióticas têm as tétrades desemparelhadas. Os grupos de cromatídes assim obtidos formam espermatídes de tamanhos variáveis, maiores ou menores do que as espermatídes dos membros paternos.*

Os espermatozóides derivados são de formas e tamanhos anômalos, geralmente gigantes.

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