The naturally mated females littered an average of 5.7 young; the embryo transfer (ET) recipients littered an average of 3.7 and 2.2 young from fresh and frozen-thawed embryos, respectively. Approximately 70% of the live-born young of natural matings survived for 8 weeks or longer, while only about 55% of the live young produced by ET survived for that period. The average litter size from ET was substantially smaller than from natural matings, and it may be that the ET mothers reared their young less well. If so, increasing the number of embryos transferred to each recipient female should increase the number of live offspring that survive.

The overall percentage of live-born from fresh embryos transferred (26%) was only slightly higher than the 20% produced from frozen-thawed embryos. However, almost twice the percentage (57%) of live young resulted from transfer of fresh embryos into recipients that littered young, compared to the percentage (31%) of young that resulted from transfer of frozen-thawed embryos into recipients that littered. This would suggest that there was a larger loss due to early embryonic mortality with frozen than with fresh embryos.

The overall efficiency of live young that survived for 8 weeks or longer from ET was 54% and 31% from fresh and frozen-thawed, respectively, compared to the number of live young produced by natural mating. These efficiencies are somewhat lower than those reported by others[2, 11, 15].

The purpose of our embryo transfers was to obtain living young from embryos recovered from a female of one genotype with respect to the immunoglobulin loci and transferred into a female of a second allotype. In that respect, the experiments were successful. We obtained a total of 75 rabbits from transfer of fresh embryos, and six from transfer of frozen-thawed embryos. All of those rabbits survived for months or even years. They have proved to be ideal experimental subjects for immunological studies to be reported elsewhere. Some of those animals produced by embryo transfer have even served as embryo donors themselves.

Acknowledgments. We should like to thank Drs C.M. Steinberg and L. Du Pasquier who read the manuscript critically. We are also indebted to Mr W. Hänggi of the animal colony at Roderdorf/SO for his invaluable help. The Basel Institute for Immunology was founded and is supported by F. Hoffamnn-La Roche&Co., Ltd, Basel (Switzerland).

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0014-4754/85/060755-02$1.50 + 0.20/0

Polyploidization and production of abnormal spermatids in *Psophus stridulus* (Orthoptera)

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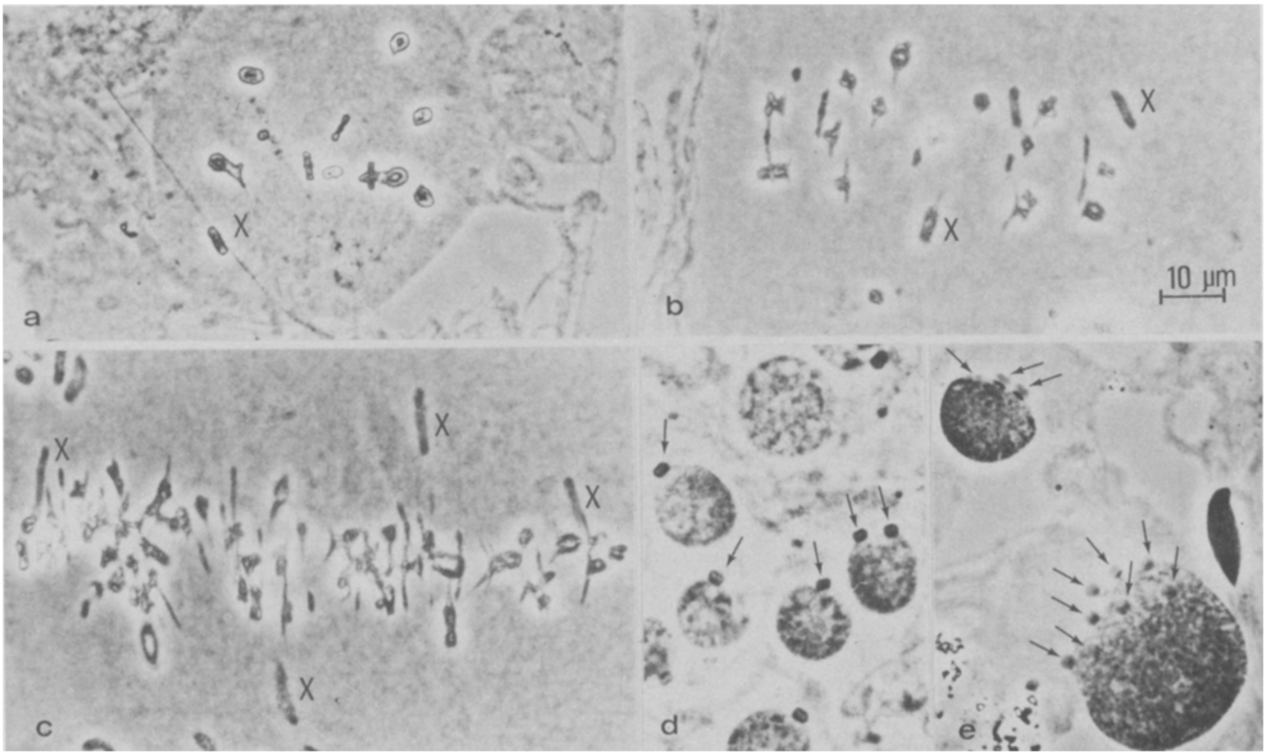
Summary. Abnormal spermatids exhibiting variation in the number of adjunct centrioles (ACs) (from two to eight) have been analyzed in a spontaneous mutant grasshopper characterized by a high tendency to form polyploid meiocytes. Results show that the observed polyploidization of these cells increases the number of abnormal gametes and, although diploid spermatids (with two ACs) are the most frequent, higher levels of ploidy are also produced. The variation in the number of ACs, the level of ploidy in the sperm and the presence of polyploid meiocytes, are topics briefly discussed.
Key words. Insect cytogenetics; spermatogenesis.

It is well known that a male, even when presenting a balanced chromosome complement, may produce sperm which in some way is not functional. During the past years, we have had the opportunity of analyzing some spontaneous mutations and chromosome polymorphisms which present a potentiality for increasing the production of abnormal spermatids in some grasshoppers[2,3]. Evidently, these analyses arise a certain interest, given that an increase in the production of non functional spermatids may have a drastic effect on the preservation of these mutations within the populations as well as on the introduction of others which appear spontaneously.

The present note deals with the production of abnormal spermatid nuclei in a male of *Psophus stridulus* presenting polyploid meiocytes. The analysis is based on only one individual, but it is necessary to bear in mind that although spontaneous polyploidization is not infrequently observed in the meiotic analysis of Orthopteroids, its low level of production does not permit us to establish a close correlation between this event and the production of abnormal spermatid nuclei. However, in the present mutant it was usual to find polyploid cells in all the follicles of the testes, favoring the possibility of establishing the correlation mentioned above.

The male used in the present study was part of a sample of 69 individuals collected in a natural population in Valle de Ordesa (Pyrenees). The testes were fixed in 3:1 ethanol:acetic acid; meiocytes were stained with lactopropionic-orcein and, in order to observe clearly both the adjunct centrioles (AC) in the spermatids and the cytoplasm of the cells, phase contrast optics were used.

The karyotype of the *Psophus stridulus* male includes 11 pairs of acrocentric autosomes plus a single, acrocentric, X-chromosome (fig., a) which implies a basic chromosome number 2n = 23. The spontaneous production of polyploid cells occurred within the cyst; consequently, different levels of ploidy were found within



Some cytogenetic aspects of the mutant. *a* Normal meiocyte at metaphase I showing 11 bivalents plus an X-chromosome. *b* A tetraploid meiocyte. *c* An octoploid meiocyte. *d*, *e* Spermatid nuclei showing different numbers of adjunct centrioles (arrows). 1 AC corresponds to a normal spermatid nucleus.

the affected cysts. Most of the polyploid primary spermatocytes were tetraploid (fig., b), while octoploid cells appeared with a much lower frequency (fig., c). Multivalent configurations were rarely found in the polyploid cells undergoing meiosis; this is interpreted as a consequence of the autonomous entity that the chromosome complements maintain within a single cytoplasm (multinucleate cells) before starting the meiotic process.
In the analysis of the abnormal spermatids, groups of them presenting the same level of ploidy were not found; this is consistent with the spontaneity of the polyploidization phenomenon. The number of ACs per spermatid, as an indicator of the ploidy level in the spermatid nuclei, varies from one (in the standard spermatids -1C-) to eight (fig., d and e, and table), two ACs being the most common number scored in the abnormal nuclei. Odd numbers of ACs (3, 5, 7) per spermatid nucleus could correspond to 3C, 5C or 7C charges of DNA per spermatid; of course, the presence of such nuclei would require the assumption of some cytogenetic explanations of its formation such as nuclear fusion or different irregular meiotic segregations which become fragile, according to the data obtained in the present case.
All the spermatids presented the ACs polarized, i.e. all the ACs were localized in a pole of the cell, irrespectively of the ploidy level. This disposition is quite orthodox, and is in line with the figures presented by other authors[3,4], but it is not the only possibility, as we have previously reported in the grasshopper *Gomphocerus sibiricus*[2].
It is fairly obvious that an unbalanced chromosome complement has the potentiality to produce abnormal meiotic products. In Orthoptera, the production of abnormal spermatids has received special attention when B-chromosomes are involved[3–5] or when interspecific hybrids are analyzed[6,7], while the influence of spontaneous mutations on this feature is less well known. Nonetheless, Nur[8], who has paid special attention to this topic, observed in the grasshopper *Melanoplus femur-rubrum* that in tetraploid follicles the telophase-I was not followed by cytokinesis, and the cells did not enter in meiosis-II and, consequently, did not form sperm. This is not the case in this mutant since at least tetraploid metaphases-II have been detected. This is interpreted as a result of the independence of the genomes in the polyploid cells, where each bivalent could separate irrespectively of the level of ploidy per cell; thus, polyploid sperm could be formed. Finally, the presence of probably 4C spermatid nuclei (with 4 ACs) can be explained by a cytoplasm which failed to divide during both meiotic divisions. However the presence of charges larger than 4C are easily explained if they come from polyploid cells.

Number of spermatids with 1–8 ACs and respective percentages: The abnormal cells include almost a quarter of the total

Number of AC	Number of spermatids		%	%
1	1814		75.52	
2		474		19.73
3		64		2.66
4		42		1.74
5		4		0.16
8		4		0.16
	1814	588	75.52	24.44
Total	2402		99.97	

1 Special thanks to Carlos García de la Vega for many helpful comments. This work was supported by C.A.I.C.Y.T. 2165/83.
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