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Arthropod Structure & Development 36 (2007) 233–243

ARTHROPOD STRUCTURE & DEVELOPMENT

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Meiotic drive by the Y-linked *D* gene in *Aedes aegypti* (L.) (Diptera: Culicidae) is associated with disruption of spermiogenesis, leading to premature senescence of spermatozoa

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Received 10 July 2006; accepted 22 November 2006

Abstract

Y chromosome meiotic drive in the mosquito *Aedes aegypti*, due to the gene *D* (Distorter) in coupling with *M* (male determination) [the *MD* haplotype], is associated with spermiogenic disruption, leading to senescence, at a rate proportionate to male excess. Spermiogenesis was compared between 'Enhanced Mutant' males with a strongly female-depleted sex ratio (8.9% females), 'Mutant' males showing a lesser degree of distortion (38.3% females), and two controls with normal sex ratios (51.2% and 49.2% females). Sections of testes dissected from mature pupae and adults aged 0, 4, 8, 12 and 16 days were examined by transmission electron microscopy. A difference between Mutant and control spermiogenesis was apparent as early as the pupal stage when some Mutant spermatids showed extra tail elements (axonemes and/or mitochondrial derivatives). The same was true of Enhanced Mutant males but to a more extreme degree. Sperm senescence was evident in Enhanced Mutant testes from day 0 of adult life but in Mutant testes not until day 4. Progressive disorganisation was associated with many loose organelles, and disturbance of the anterior–posterior axis of gamete differentiation within the testis. Degenerative changes of a similar kind in the controls did not become apparent until day 8. These findings are discussed with respect to other characteristics of this meiotic drive system, in terms of a theory of inhibition of reduction division in spermatogenesis associated with fragmentation of the X chromosome, leading to the formation of a restitution nucleus as early as metaphase 1.

Keywords: Mosquito; Y-chromosome; Sex ratio; Spermatocyst; Spermatid; Axoneme; Mitochondrial derivative; Restitution nucleus

1. Introduction

Sex in the mosquito *Aedes aegypti* is controlled by an XY mechanism, the genotypes of males and females being *M*/*m* and *m*/*m* respectively (Newton et al., 1974). Y chromosome meiotic drive in the mosquito *Ae. aegypti*, due to the gene *D* ('Distorter'), inherited in coupling with the male-determining factor *M* [the *MD* haplotype], produces male-biased sex ratios. Cytogenetic evidence has shown that the sex ratio bias is quantitatively associated with preferential X chromosome breakage during meiosis, at or before diplotene, leading to the loss of potential X spermatozoa. A positive correlation was observed between the extent of breakage and the degree of sex ratio distortion (Newton et al., 1976, 1978a). It was clear, however, that the differential loss of potential X spermatozoa was not the only outcome of preferential chromosome breakage. Two further observations were made by Newton et al. (1976): (1) the presence of acentric X chromosome fragments remaining attached to segregating Y chromosomes; (2) evidence (from comparing X chromosome breakage rates and sex ratios) that some broken X chromosomes were proceeding further

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doi:10.1016/j.asd.2006.11.003

in spermatogenesis than the first meiotic division. It was subsequently found that an extreme level of sex ratio distortion was associated with gross enlargement of some spermatozoa with up to four times the normal DNA content (Newton et al., 1978b).

Spermiogenesis in *Ae. aegypti* can be followed, using transmission EM of the sectioned testis, in all its stages from spermatogonia to spermatozoa. The testes are spindle-shaped, about 0.4 mm in length and 0.1 mm in greatest breadth (Christophers, 1960), banded transversely. The bands indicate regions of spermatocysts within which clones of germ cell have developed to various stages, with spermatogonia at the proximal end and mature spermatozoa at the posterior (distal) end, and in between several bands of primary spermatocytes, secondary spermatocytes and spermatids. Shortly after adult emergence from pupae, spermatozoa are released into twin vasa deferentia, to be stored in a common seminal vesicle. Sperm production begins in the pupae and may continue in the adult although the rate at which new sperm are produced is negligible after 1–2 days of adult life (Jones and Wheeler, 1965).

Spermiogenesis in *Ae. aegypti* has been reviewed from the work of various authors by Clements (1992, pp. 333–339). The early stage in the formation of the tail is associated with the accumulation of a group of mitochondria at the posterior end of each immature spermatid. These mitochondria fuse to form a structure called the nebenkern destined, in normal spermiogenesis, to divide to form two mitochondrial derivatives. Meanwhile a single flagellum (axoneme) originates from the posterior pole of the nucleus and passes between the two recently formed mitochondrial derivatives. As the axoneme with its associated mitochondrial derivatives continues to elongate, each mitochondrial derivative is replaced progressively by a condensation of paracrystalline bodies until these occupy the mitochondrial derivatives almost entirely.

Y-linked meiotic drive has also been observed in the Mediterranean fruit fly *Ceratitis capitata* (Wied.). In a study of spermiogenesis in this species, using transmission electron microscopy (TEM), Shahjahan et al. (2006) observed a direct association between excess male production and loss of a proportion of spermatids. The latter was established by counts made on individual spermatocysts within the testis. Loss of spermatids was accompanied by the presence in some of those remaining of characteristic morphological abnormalities in the form of extra tail elements (axonemes) and/or mitochondrial derivatives.

The present TEM study of spermiogenesis in *Ae. aegypti* was designed to compare different stages of spermatid/sperm tail development associated with two different degrees of meiotic drive in this species. This approach was followed because of the impossibility of making accurate counts of spermatids per spermatocyst (as in *C. capitata*), the cyst walls being unstable in *Ae. aegypti*. Different degrees of meiotic drive were achieved by placing the *MD* haplotype in conjunction with a genetic background showing different levels of sensitivity to its driving effect. Such males (with *MD* present and active), producing an excess of males in their progeny, were compared with matched control males (with the same genetic background but with *MD* absent), which generate the sexes in equal proportion.

2. Materials and methods

2.1. Strains

The three strains of *Ae. aegypti* T8, 68Syn1a and 3M, from which crosses were made to generate the males used for the study, were maintained at 28 ± 1 °C and $75 \pm 5\%$ RH. 3M is a substrain of TPM (= RED), a triple-marked strain obtained from Dr. L. Mustermann at the University of Notre Dame, Indiana, USA. The X chromosomes of TPM (and thus 3M) are sensitive to *MD* (Hickey and Craig, 1966; Wood and Ouda, 1987). T8, which is homogeneous for *MD*, is a strain of Trinidad origin long maintained at the University of Manchester. 68Syn1a is a strain constructed by combining X chromosomes from an Australian strain, Thursday Island (obtained from Dr I. Fanning at the Institute of Medical Research, Brisbane, Australia) with Y chromosomes from 3M, in which *D* is absent. The X chromosomes of Thursday Island had been previously selected for sensitivity to T8 *MD*, using a breeding scheme reported by Owusu-Daaku et al. (1997).

2.2. Production of Mutant and Enhanced Mutant males

F1 males, showing either moderate drive (Mutant), or more pronounced drive (Enhanced Mutant), were generated from the crosses indicated in Table 1, from 20 females mated with 20 males, yielding about 1000 progeny per blood meal. The 'Control' in each case came from the reciprocal cross, matching the breeding scheme of Newton et al. (1976). Half the F1 eggs produced from these crosses were bred to F2 where the sex ratio was assessed. The remaining F1 eggs were hatched, from which males were randomly selected, either as pupae or as adults at 0, 4, 8, 12 and 16 days after emergence, for ultrastructural examination.

2.3. Ultrastructural examination by transmission electron microscopy

Individuals for examination were immobilised by chilling before removing the testes. Dissections were carried out in Ringer solution on a slide, under a stereo microscope. The pleuron was broken laterally between the 4th and 5th

Table 1
Crosses used to generate hybrid males exhibiting two degrees of Y-linked meiotic drive, reflected in male-biased F2 sex ratio, compared with controls derived from reciprocal crosses

Cross	F1 description	F2 sex ratio (% ♀)
3M♀×T8♂	Mutant	38.3
T8♀×3M♂	Control 1	49.2
68Syn1a♀×T8♂	Enhanced Mutant	8.9
T8♀×68Syn1a♂	Control 2	51.2

Each sex ratio was based on a count of at least 1000 pupae.