SPERMIOGENESIS AND MEIOTIC DRIVE IN *AEDES AEGYPTI*

K.O. OWUSU-DAAKU, R.D. BUTLER, R.J. WOOD
School of Biological Sciences, University of Manchester, Manchester, M13 9PT, UK

Abstract—We recognise that insecticides must continue to play a major role in most control programmes against insect pests or vectors, particularly when there are emergencies which cannot wait for alternative means of control to be developed. We believe genetic methods should be given serious thought and investment, in any long term strategy. This opinion is strengthened against a background of (i) expanding insecticide resistance (ii) tightening regulation of insecticide use. (iii) multiple drug resistance in vector borne parasites and (iv) considerable progress made in genetic engineering and recombinant DNA research.

In our recent work on spermiogenesis and meiotic drive in the mosquito, *Aedes aegypti*, we have established that some strains carry the distorter gene *D*on the Y chromosome which causes the production of more males than females, the proportion of females being below 12% in some cases. Any means of producing fewer blood engorging females must be good news. Electron microscopic studies on testes reveal evidence of massive degeneration of spermatozoa, reduction in their numbers within cysts and considerable degree of malformation, and early senescence in strains which respond to the effect of the meiotic drive by *D*. These features are evident in the testes of emergent males, which will not be ready to mate till 48 hours later (Christophers, 1960; Clements, 1992).

Meiotic drive has been mentioned as one possible vehicle by which genes altered through recombinant DNA technology could be introduced into the populations (Eggleston,1991).

We would be the first to recognise the difficulties that lie ahead, in any attempt to exploit the potential of genetic control commercially The fact that most of the recommendations of the Rockefeller Foundation committee of experts (Hoy and McKelvey,1979) still remain unfulfilled a decade and a half after they were made, in spite of a considerable increase in knowledge of gene technology puts aspirations for genetic control in perspective. At the same time we believe that any progress in this direction is better than none at all.

INTRODUCTION

Ever since man decided to take control and dominate other organisms in their shared environment, he has gone to great lengths in protecting the organisms he considers beneficial and waging war against harmful ones. In the category of the harmful, fall the mosquitoes, vectors of diseases. Among these are *Anopheles spp.* which carry various forms of the protozoan *Plasmodium spp.*, causing malaria, *Culex spp.* which carry the microfilarial worm *Wuchereria bancrofti*, producing the grossly deforming elephantiasis and *Aedes spp.* which carry the arboviruses responsible for of yellow fever and dengue fever, including its often fatal haemorrhagic form. Altogether, about 2/5 of the world's population (2.1 billion) are potentially at risk from mosquito transmitted diseases. In some parts of Sub-saharan Africa, 1 in 10 infant deaths and 1 in 4 deaths of children under the age of 4 years are attributed to malaria. (Fullick and Fullick, 1993). Annually 400 million people are at risk, of whom 270 million are infected, causing 2 million deaths. To this enormous burden of human suffering and death must be added the cost of the treatment of the sick, the loss to economic development and the cost of control programmes (Laird and Miles, 1983; 1985). Eliminating the vectors of such diseases is the most radical solution. Drug treatment only eliminates the parasite, leaving the insect to continue biting.

A number of approaches have become available in vector control, mainly chemical, directed against adults or larvae. Other methods include (a) biological control using natural predators like the larvivorous fish as *Gambusia affinis*, or natural toxins such as that produced by the bacterium *Bacillus thuringiensis* (b) environmental control by removal of breeding sites and public education.

In as much as chemical control is the most tested, the most frequently used and the most effective method in acute situations, it will continue to play a major role in any control programme. There are, however, considerable problems associated with the use of insecticides (Hoy and McKelvey,1979; Laird and Miles, 1983 and 1985 ;Eggleston,1991). The more important of these are (i) the evolution of insecticide resistance to all four major classes of insecticide - organochlorines, organophosphates, carbamates and pyrethroids, (ii) the high cost involved in developing and

Proceedings of the First International Conference on Urban Pests.
K.B. Wildey and Wm H.Robinson (editors). 1993

registering new insecticides (iii) increasing legislation over their use and (iv) growing concern over the environmental impact of toxic residues (Eggleston,1991).

Various genetic control methods have been shown to have potential against vectors (WHO, 1964; Hoy and McKelvey, 1979; Curtis and Graves, 1988), yet none to date has been exploited commercially, except for the Sterile Insect Technique (SIT). Genetic control has been defined as "the use of any condition or treatment that can reduce the reproductive potential of noxious forms by altering or replacing hereditary material." (WHO,1964). Curtis and Graves in (1988) reviewed six possible approaches, one of which was meiotic drive, the subject of this paper.

Meiotic drive refers to a mode of genetic inheritance in which a heterozygote generates gametes with one of the two alternatives(alleles) in excess; a deviation from the expected 1:1 ratio, of Mendel's 1st law. Meiotic drive is not a new concept, having been known since the '20s, and finally defined by Sandler and Novitski (1957). It was comprehensively reviewed by Zimmering *et al.* (1970). It is probably the most reviewed genetic control concept after the SIT. It was independently described in *Aedes aegypti* by Craig and others (1960) and Wood (1961). Views, about its potential for insect control and even how it is thought to operate are far from being in unison (Whitten, 1970; Pal and Whitten, 1974; Lyttle,1977; Hurst and Pomiankowski, 1991; Frank, 1991; Johnson and Wu, 1992). Nevertheless there seems to be agreement that the phenomenon does have a potential role to play in insect control in association with other methodologies, particularly in the direction of genetic engineering (Morrison, *et al.* 1989; Eggleston, 1991). We therefore believe it is a subject which ought to be researched and understood better.

The gene in *Ae. aegypti*, known as *D (Distorter)* is located on the Y chromosome, causing excess production of males (Wood and Newton, 1991).In view of the fact that *D* appears to suffer no selective disadvantage in terms of fertility, it is able to pass from generation to generation. It is truly a "selfish gene" (Dawkins, 1976; Johnson and Wu, 1991; Wu and Hammer, 1991). Being Y-linked, close to the male determining region *M*, it causes loss of X chromosomes. Physical evidence of this was reported by Newton *et al* (1976), who observed the physical break up of the homologous X chromosome at meiotic metaphase. Consequently, in the resulting spermatozoa, the, Y-bearing sperms are in the majority and the sex ratio is biased towards male excess. Pearson and Wood (1980) produced selected lines supporting less than 2% females.

The present study was designed to investigate, by electron microscopy the effect of D in spermiogenesis and to observe at what stage in the process, the X spermatozoa degenerated. As a prelude, it was necessary to isolate a cross which would produce a substantial degree of sex ratio distortion, ie. one with *D* Y-chromosome coupled with X-chromosome highly sensitive to *D*.

MATERIALS AND METHODS

Nine laboratory and twelve wild strains of *Ae. aegypti* were studied (Fig.1). Of the laboratory strains, three had been established for over 30 years and these are the *Trinidad, (T8), Rock (R)* and the *Triple - marked (3M)*. The remainder have been for about 10 years- *Townsville, Thursday Island (Th.I), Penang,* and *Bangalore, Hainang* and *Venezuela.* Of the 12 wild strains, 11 were Ghanaian, collected by Owusu-Daaku in the summer and early autumn of 1991, the other a Ugandan strain collected in 1990 and named *Entebbe(E)*. The Ghanaian strains were representative of the country and include coastal strains - *Darkuman (DRK), Ghana International(GIS), Elmina (ELM) and* Sekondi (SEK)*; forest strains - Asuoyeboa (ASU), Suame (SUA) Suhum (SUM), Ekyi Amanfrom (EKY)* and *Nkawkaw (NKW)*. There was also one savanna grassland strain, *Wa (WA)* and one strain from a transition zone (between forest and savanna) *Kintampo (KIN)*. (Fig. 2) Taking together, the strains represent a global tropical sample (Fig.1).

Laboratory maintenance of Ae. aegypti

Stored eggs on filter paper cones were hatched in hay infusion. Larvae were fed on crushed dog biscuits. Pupae were sexed into male and female according to size (females are bigger), and each sex transferred to fresh water for emergence within netting cages (25cm × 25cm × 25cm). All stages were kept in insectaries with room temperature around 25°C ± 1 and relative humidity of 75% ± 5 Mass or single-pair (*SPM*) crosses were made within or between strains.

Proceedings of the First International Conference on Urban Pests.
K.B. Wildey and Wm H.Robinson (editors). 1993

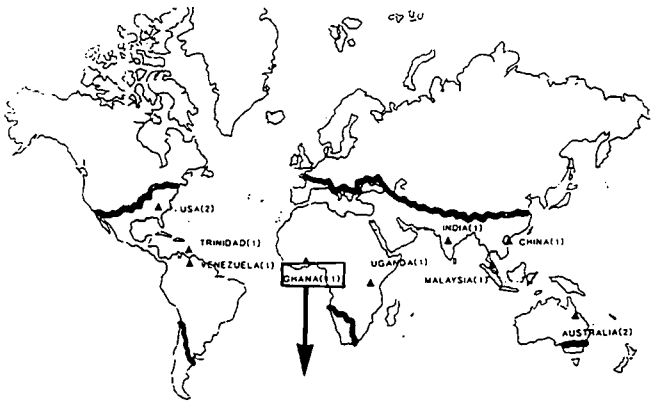

FIG.1 Global limits of Aedes aegypti and countries of strains used

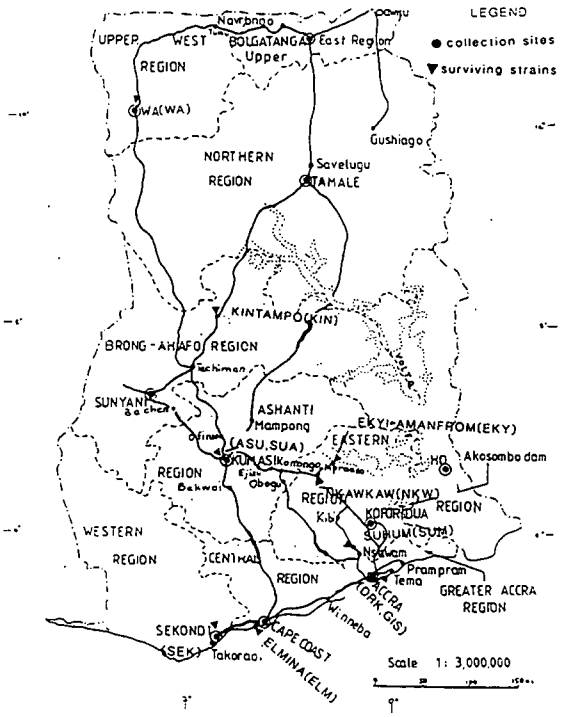

FIG. 2. Map of GHANA showing sites of collection of Aedes aegypti.

Meiotic drive

In order to assess whether any particular strain carried the *D* gene the males were crossed to females of a strain known to have an X chromosome sensitive to *D*. The F1 was then inbred to give F2. A male - distorted sex ratio in F2 indicated the presence of *D*. To assess whether any particular strain was sensitive to D, the females were crossed to males known to carry *D*. The F1 was then inbred to give F2. A male - distorted sex ratio in F2 indicated sensitivity to *D*. In fact, at the beginning, little was known about the characteristics of most strains , so reciprocal crosses were made between most of them, and information was thereby gradually built up.

Maintenance of Distortion

Having established which crosses yielded distorted sex ratios and which did not, four *Th.I*♀ × *T8*♂ crosses were followed through to F10 generation. The Th.I females used for the crosses were

taken from lines which had been previously selected because of the sensitivity of their X chromosomes.

Spermiogenesis

Once a particular cross has been followed through and the sex ratio analysed at F2, previously stored F1 eggs were hatched, the larvae allowed to pupate, and pupae sexed. Male adults were dissected and testes of individuals at 0, 4, 8, 12, and 16 days were fixed and thin- sectioned for examination by transmission electron microscopy. . A reciprocal cross was similarly treated and acted as a control.

RESULTS

Meiotic drive assessment

Mass crosses in either direction between *Trinidad* (also called *T8*) *Entebbe, Triple-marked* (also called 3M) , *Rock* and *Thursday Island,* abbreviated in text as *T, E, 3, R and Th.I* respectively, gave sex ratios indicated in Table 1. The results revealed that only in the case of *T* were the X chromosomes sensitive to Y chromosome of the same strain.

This was also true of all the many other crosses carried out (data not reported here), with the possible exception of *Venezuela*. The X chromosomes of *E* and *3* were both sensitive to drive by the *T* Y chromosome. Those of *3* and *R* were both sensitive to drive by *E. Those of 3* and *T* were both sensitive to drive by *R. 3* was thus uniformly sensitive to drive but showed no drive itself. Equivalent single-pair (SPM) matings showed similar results although, as expected, there was higher variability between families than between replicate mass crosses . As few as 13.9% females were observed in F2 from cross *E*♀ × T♂ (ie. from *EYTX* males), as few as 15.0% females in F2 from cross *3*♀ × *R*♂ (ie. *from* 3XRY males) and as few as 11.1% females in F2 from cross *Th.I*♀ × *T*♂(ie. from cross Th.IXTY *males).*

It is an invariable rule that when there is distortion in one direction,the reciprocal cross shows no distortion at all. (TABLES 1, FIGS. 3A-C). The greatest distortion observed overall in this series of experiments was from males inheriting the *R Y* chromosomes coupled with *T X* chromosomes (31.07 +/- 2.08♀%).

Maintenance of distortion

A selection experiment(not reported here) produced some *Th.I* lines which appeared to be more sensitive to *D. These were 14Th.I, 46Th.I, 68Th.I and 75Th.I. In the cross 14Th.I*♀ × *T*♂, the mean % over the 10 generations was 29.77 (Table 2), in *46Th.I*♀ × *T*♂, 37.94 (TABLE 3), in *68Th.I*♀ × *T*♂, 28.45 (TABLE 4) and in *75 Th.I*♀ × *T*♂, 33.98 (Table 5). Significant sex ratio distortion in most generations in each of the four crosses being followed. Two further interesting points are, (i) the four lines maintained their order of distortion over the generations *68, > 14, > 75 > 46.* (ii) that if variation in degree of distortion between generations was compared between crosses, a fairly consistent pattern was observed; eg. there was an increase females in F3 and F4, followed by a decrease in F5 (Fig. 3D).

Spermiogenesis

We report here the result of examining by transmission electron microscopy, the testes of newly emerged males (0 day).These are the testes of F1 males, sibs of those which had yielded a highly distorting F2 ratio (% ♀= 11.05) from the cross *68Th.I*♀ × *T8*♂ (Plate 2) and compared with reciprocal cross of *T8*♀ × *68Th.I*♂ *F1* (Plate 1). 2A shows a cyst in which there is massive degeneration of spermatozoa. 2B is a micrograph of a field showing both mature and immature sperms undergoing degeneration. 2C, E and F show an emptying cyst with a vacuole in the testicular wall where sperms are digested. As cysts empty they are filled with debris as well some

Table 1. Mean percentage female and standard error in four strains and in F2 from mass crosses between these strains, based on at least six replicated samples. the X and Y constitution of the male parents of the samples (F1 males in the case of crosses) is indicated.

Y CHROMOSOME	X CHROMOSOME			
	T8 (T)	**ENTEBBE (E)**	**3M (3)**	**ROCK (R)**
T8 (T)	41.95+/-1.23*	7.97+/-2.63*	38.68+/-0.93*	47.72+/-1.64
ENTEBBE (E)	51.45+/-2.01	49.12+/-0.63	38.50+/-1.09*	40.90+/-2.33*
3M (3)	49.33+/-O.63	48.55+/-0.80	48.87+/-0.48	50.00+/-0.45
ROCK	31.07+/-2.08*	48.22+/-1.17	36.17+/-1.34*	49.67+/-1.23*

*Sex ratio significantly different from 1♀ : 1♂

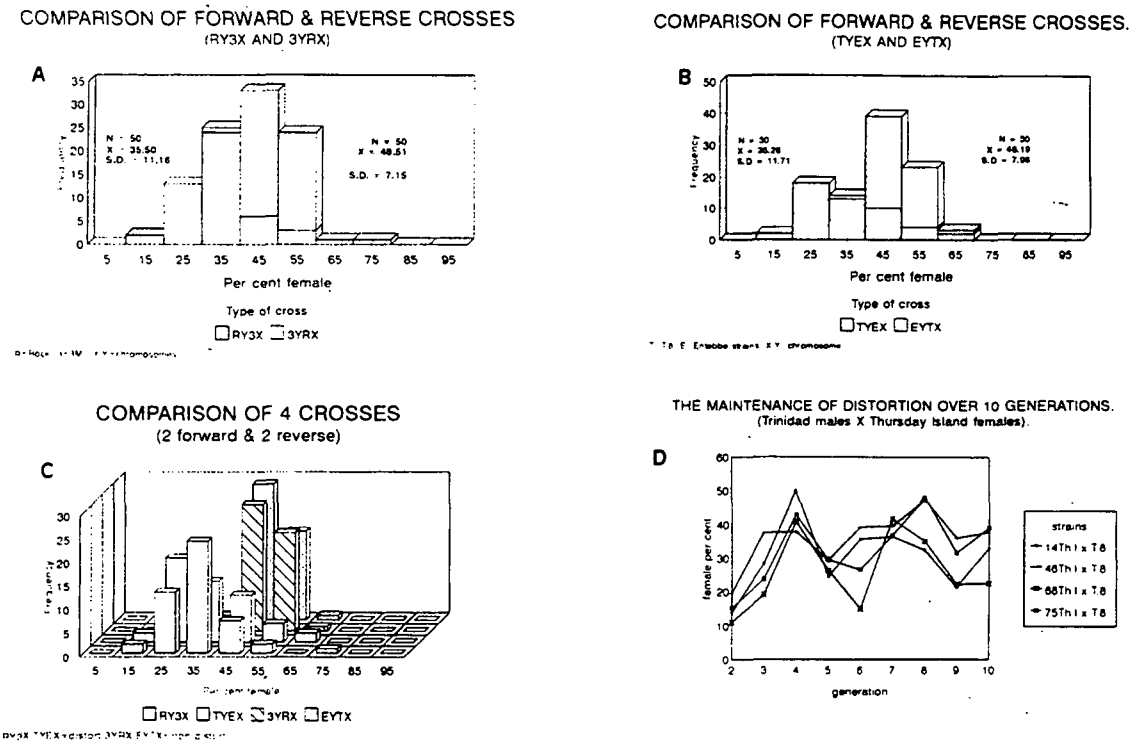

Figure 3 (a)–(d)

delimiting membranes, waste bags within which sperm degradation takes place. 2D is a close up view of another common feature of the distorter testis, the presence of multiple organelles, and demembrination of others, as well as loss of organelles (not shown in 2D).

In contrast, the reciprocal cross *T8♀ × 68Th.I♂ F1,* hardly shows any malformations at this early age. A transverse section of a cyst (1A) with 493 spermatozoa, close to the full complement of about 500 sperms,(Roosen-Runge,1977). Presumably, the full complement is 512. The number having to be in agreement with the fact that germ cell divisions must be a clone multiple of 2 (Phillips, 1974; Wandall, 1986). Mature sperms have fully condensed nuclei (1B) and a flagellum consisting of a pair of crystalline mitochondrial derivatives, which sandwich the second organelle, the axoneme,for most of the length of the flagellum. The axoneme consists of a set of microtubules a central tubule, surrounded by nine inner doublets and nine outer peripheral singlets, hence the configuration of 9 + 9 + 1 (1C, D, E and F). In a longitudinal section the nucleus appears bullet shaped and capped anteriorly with a translucent acrosome (1G). In contrast to the distorter case, cells within a

Table 2. Maintenance of distortion over 10 generations
14Th.1♀ × T8♂

Cross	Total	♀	♂	X^2	%♀
F2	237	33	204	123.38	13.92*
F3	148	42	108	27.68	28.38*
F4	198	99	99	0.00	50.00
F5	318	78	204	82.53	24.53*
F6	790	217	573	160.43	27.47*
F7	296	108	188	21.62	36.49*
F8	292	95	197	35.63	32.53*
F9	526	113	413	171.10	21.48*
F10	266	88	178	30.45	33.08*
Mean	29.77				
Variance	91.94				
Std Deviation	9.59				
S.E.M	10.22				
CV	0.32				
%♀ Range	13.92 - 50.00				
*	Distortion				

Table 3. Maintenance of distortion over 10 generations
46Th.1♀ × T8♂

Cross	**Total**	♀	♂	X^2	%♀
F2	481	94	387	178.48	19.54*
F3	226	85	141	13.88	37.61*
F4	414	158	256	23.20	38.16*
F5	265	78	187	44.83	29.43*
F6	490	232	258	1.38	47.35
F7	363	144	219	15.50	39.67*
F8	251	119	132	0.67	47.41
F9	238	106	132	2.84	44.54
F10	135	51	84	8.07	37.78*
Mean	37.94				
Variance	70.63				
Std Deviation	8.40				
S.E.M	7.85				
CV	0.22				
%♀ Range	19.54 - 47.41				
*	Distortion				

Table 4. Maintenance of distortion over 10 generations
68 Th.I × T8

Cross	**Total**	♀	♂	X^2	%♀
F2	190	21	169	115.28	11.05*
F3	161	31	130	60.88	19.25*
F4	276	113	163	9.06	40.94*
F5	501	132	369	112.11	26.35*
F6	392	144	248	27.59	36.73*
F7	194	81	113	5.28	41.75*
F8	322	113	209	28.62	35.09*
F9	372	83	289	114.08	22.31*
F10	319	72	247	96.00	22.57*
Mean	28.45				
Variance	101.07				
Std Deviation	10.05				
S.E.M	11.23				
CV	0.35				
%♀ Range	11.05 - 41.75				
*	Distortion				

Table 5. Maintenance of distortion over 10 generations
75 Th.I♀ × T8♂

Cross	TotaL	♀	♂	χ^2	%♀
F2	184	28	156	89.04	15.22
F3	164	39	125	45.10	23.78*
F4	380	163	217	7.67	42.89*
F5	571	169	402	95.08	29.60*
F6	597	231	366	30.53	38.69*
F7	301	111	190	20.73	36.88*
F8	463	223	240	0.62	48.16
F9	382	121	261	51.31	31.68*
F10	480	187	293	23.41	38.96*
Mean	33.98				
Variance	90.74				
Std Deviation	9.53				
S.E.M	10.08				
CV	0.28				
%♀ Range	15.22 - 48.16				
*	Distortion				

cyst tend to be at the same level of development (1H, I and 2B). We conclude that meiotic drive leads to malformation and rapid degeneration of many spermatozoa.

DISCUSSION

We have shown that sex ratio distortion persists to a significant degree for 10 generation when the *Trinidad Y*-chromosome is paired with X chromosome of the Australian *Th.I* strain. The result is artificial in so far as the *T Y*- chromosome is not having to compete with the native one. There is now need for an experiment to ascertain whether the *T Y*- chromosome would compete effectively with the native *Th.I Y* chromosome. According to theory the former should replace the latter because it will leave more copies of itself (Hamilton, 1967; Wu and Hammer, 1991). It remains to be demonstrated, however, whether the maximum possible load exerted (reduction of females from 50% to about 30%) is sufficient to depress fertility significantly. With other combinations of strains it may be possible to do better.Thus the use of meiotic drive as an agent of population suppression remains theoretically possible but unproven (Wood and Newton,1991).

Its true potential may be in combination with other methods (Rai et al, 1970; Suguna et al, 1977; Curtis,1978). Suguna *et al.* (1977) showed that population suppression could be achieved in field cage experiments by release of males carrying *D* combined with two reciprocal translocations. Recombinant DNA studies may be opening up further possibilities to enhance the action of *D* (Morrison *et al.*, 1989; Eggleston, 1991).

The work on spermiogenesis supports the view that the meiotic drive system is the result of a post meiotic failure of the X bearing sperm to go through successful fertilisation, (Wood and Newton, 1991). This was earlier noted in relation to meiotic drive at the *SD* locus in *Drosophila* (Novitski et. al., 1965; Peacock *et. al.*, 1972). We believe that meiotic drive has sufficient potential as a vehicle of mosquito control for pursuing further work on the concept, particularly in relation to recent advances in gene technology. The rapid progress in molecular genetics accelerates by the day.

Clearly there appear to be several possibilities for manipulating meiotic drive:

1) To enhance the action of *D* to produce "super drive", a class of Y chromosomes which will drive against any type of X chromosome from the wild. 2) To make the action of *D* conditional upon a defined environmental stimulus, eg. associate it with a heat sensitive promoter. Its action can then be repressed for the convenience of laboratory rearing, and switched on before release in the field. 3) To link *D* absolutely firmly to *M* so there is no possibility of X drive. 4) To link *D* with a "useful" gene (eg. one which inhibits transmission of defined disease). 5) To transfer the *D* gene from *Aedes* to *Anopheles* mosquitoes. To advance in any of these directions will require fundamental research on meiotic drive and how it works.

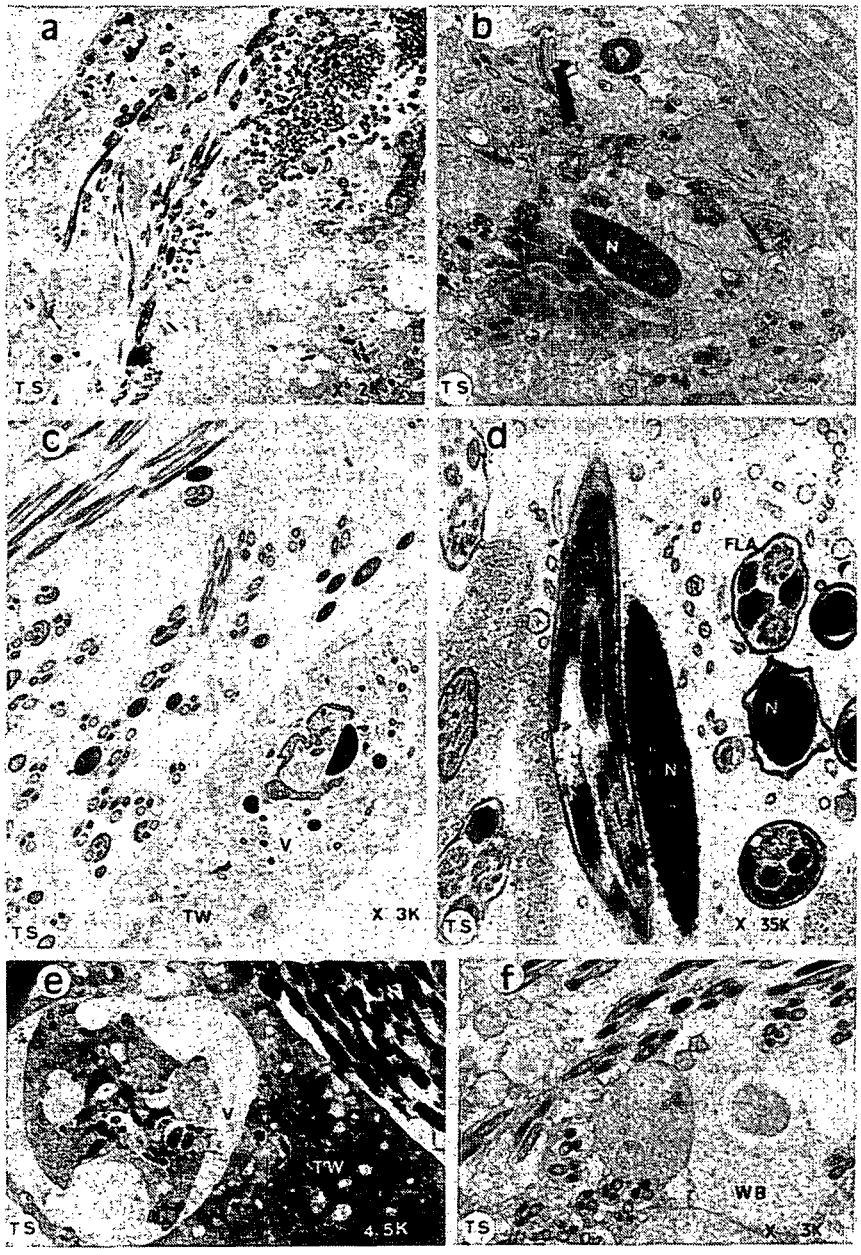

PLATE 1. A. A cyst with degeerating spermatozoa. B. Mature and immature spermatozoa degenerating together. C&E. An emptying cyst, with a vacuole in the testicular wall where degeneration of sperms occur. D. A field showing a nucleus devoid of a membrane and flagella with duplicated axonemes with inadequate mitochondrial derivative complement. F. A field filled with waste bags and degenerating spermatozoa.
Abbreviations: N. nucleus, TW. testicular wall, V. vacuole, WB. waste bag.

Clearly, the usefulness of genetic manipulation as an approach to the control of insects has to be carefully assessed, as there will be practical and perhaps ethical difficulties to overcome (Eggleston, 1991). The cost of a misfired genetic manipulation may perhaps be put on the same scale as a radiation fall-out. Each potential method must be tested carefully. Even a simple release of *Ae.aegypti* from one part of the world to another runs the risk of introducing the vector for new disease transmission. There are no "quick fix" solutions to genetic control but as a Ghanaian proverb aptly puts it,"There is a world of difference between what I began and failed and what I did not begin at all for fear of failure," Or as the English say,"He who makes no mistakes never makes anything". Only by being adventurous with our science can we keep one step ahead of the insects.

Proceedings of the First International Conference on Urban Pests.
K.B. Wildey and Wm H.Robinson (editors). 1993

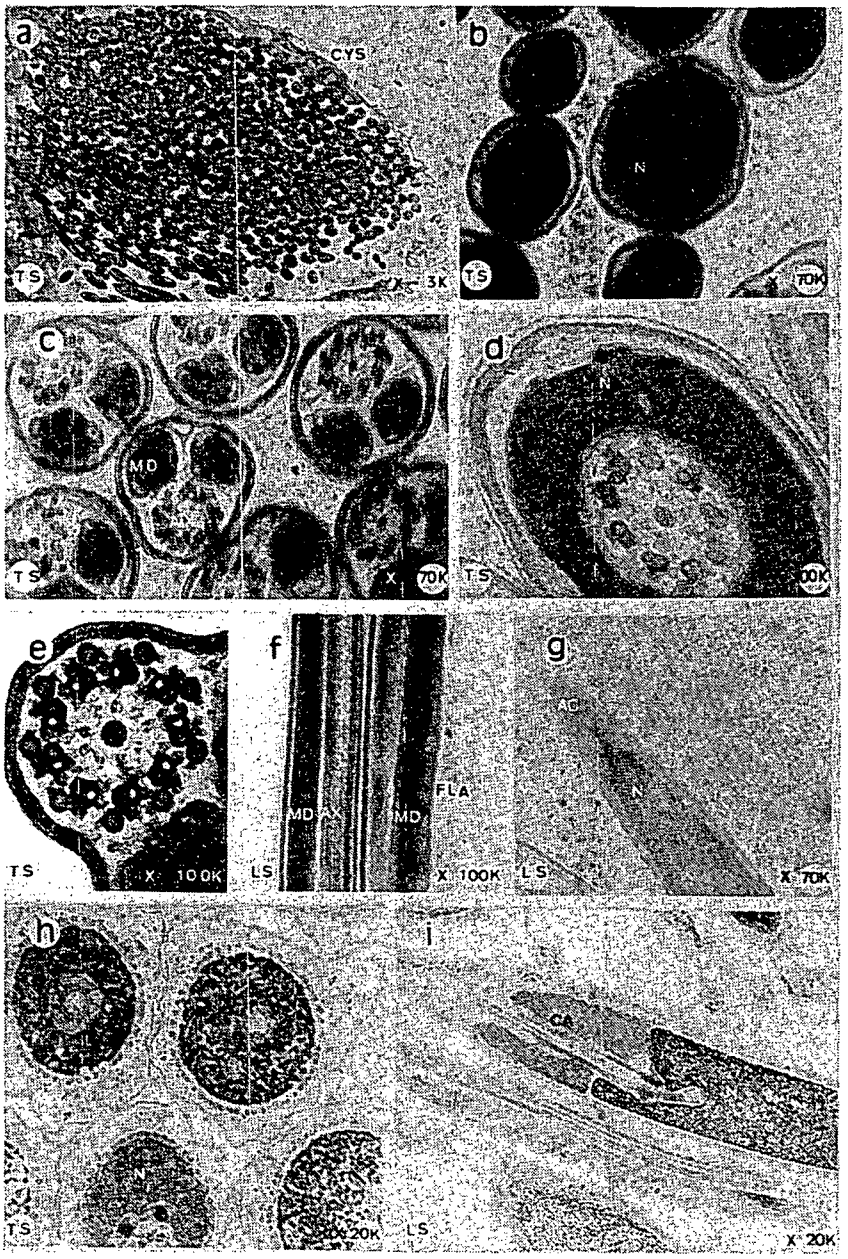

PLATE 2. A. A cyst with its full complement of spermatozoa. B. A close up of nuclei with membranes intact. C. A field of normal flagella, each with a pair of mitochondrial derivatives and an axoneme. D. A close up view of the junction between the posterior end of the nucleus and the anterior end of the axoneme. E&F. A close up TS and LS views of the flagellum. G. Anterior end of nucleus showing acrosome. H.&I. A TS and LS orientation of immature spermatozoa at the same level ofdevelopment.
Abbreviations: AC. acrosome, AX. axoneme, CA. centriole adjunct, CYS. cyst, FLA. flagellum, MD. mitochondrial derivative.

ACKNOWLEDGEMENTS

This work comes out of a three- year support grant for a Ph.D programme, by the *Government of GHANA*. The authors also acknowledge a one-off grant from *The Lindeth Charitable Trust, U.K.*, under "Grant for Research in African Terrestial Ecology."

REFERENCES

Christophers, S.R., (1960) *Aedes aegypti* : The yellow fever mosquito; its life history, bionomics and structure, 1st edn. Cambridge university press, London. pp. 739.

Clements, A.N., (1992) The biology of mosquitoes, 1st edn, vol 1. Chapman and Hall, London. pp. 509

Craig, G.B., Hickey, W.A., Vandehey, R.C., (1960) An inherited male producing factor in *Aedes aegypti. Sci 132:1887-1889*

Curtis, C.F., Graves PM (1988) Methods for replacement of malaria vector populations. Journal of tropical medicine and hygiene *91:43-48*

Curtis, C.F., (1978) Translocations, hybrid sterility, and the introduction into pest populations of genes favourable to man: in Genetics in relation to Insect management; Hoy and McKelvey(eds), The Rockefeller Foundation 1979.

Dawkins, R., (1976) The selfish gene. Oxford university press, London. pp. 224.

Eggleston, P., (1991) The control of insect-borne disease through recombinant DNA technology. Heredity **66**:161-172

Frank, A.S., (1991) Divergence of meiotic drive - suppression systems as an explanation for sex biased hybrid sterility and inviability. Evolution **45**(2 Suppl.):262-267

Fullick, A., Fullick P., (1993) Tropical diseases. New scientist February(Inside science. **59**:1-4)

Hamilton, W.D., (1967) Extraordinary Sex ratios. Science **156**:477- 488.

Hoy, M., McKelvey, J.J., (eds) (1979) Genetics in relation to insect management. The Rockefeller foundation publication pp. 179

Hurst, L.D., Pomiankowski, A., (1991) Sex ratio bias may account for unisexual sterility in hybrids: explanations of Haldane's rule and related phenomena. Genetics **128**:841-858

Johnson, N.A., Wu, C.I., (1992) An empirical test of the meiotic drive model of hybrid sterility: sex-ratio data from hybrids between *Drosophila simulans* and *Drosophila sechellia. Genetics 130:507- 511*

Laird, M., Miles, J.W., (eds) (1983) Integrated mosquito control methodologies, vol 1. Academic press, London, New York. pp.369

Laird, M., Miles, J.W., (eds) (1985) Integrated mosquito control methodologies, vol 2. Academic press, London, New York. pp. 444

Lyttle, T.W., (1977) Experimental population genetics of meiotic drive system I. Pseudo -Y chromosome as a means of eliminating a cage population of *Drosophila melanogaster. Genetics 86:413-45*

Morrison, A.C., Eggleston, P., Crampton J.M., (1989) Genetic transformation of the mosquito *Aedes aegypti* by micro-injection of DNA. Medical and veterinary entomology **3**:1-7

Newton, M.E., Wood, R.J., Southern, D.I., (1976) A cytogenetic analysis of meiotic drive in the mosquito *Aedes aegypti* (L). Genetica **46**:297-318

Novitski, E., Peacock, W.J., Engel, J., (1965) Cytological basis of sex ratio distortion in *Drosophila Pseudoobscura. Sci. 148:516-517*

Pal, R., Whitten, M.J., (eds) (1974) The use of genetics in insect control. Elsevier scientific publishing company, pp. 241

Peacock, W.J., Tokuyasu, K.T., Hardy, R.N., (1972) Proceedings of the international symposium: **The genetics of the spermatozoon** Pergamon press, London. pp. 247-268

Pearson, A.M., Wood, R.J., (1980) Combining the meiotic drive gene D and the translocation T1 in the mosquito *Aedes aegypti* L. 1. sex ratio distortion and fertility. Genetica **51**:203-210

Philips, D.M.,(1970) Insect sperm: Their structure and morphogenesis. Journal of Cell Biology **44**:243-277

Rai, K.S., Lorimer, N., Hallinson, E., (1974) The current status of genetic methods for controlling *Aedes aegypti* in the use of genetics in insect control; Pal, R., Whitten, M.J., (eds) 1st edn. Elsevier scientific publishing company, Amsterdam, New York, London,

Roosen-Runge, E.C., (1977) Spermatogenesis in animals, 1st edn. Cambridge university press, London. pp. 214

Sandler, L., Novitski, E., (1957) Meiotic drive as an evolutionary force. The American naturalist **91**:105-110

Suguna, S.G., Wood, R.J., Curtis, C.F., Whitelaw, A., Kazmi S.J., (1977) Resistance to meiotic drive and the MD locus in an Indian wild population of *Aedes aegypti. Genet. Res. 29:123-132*

Wandall, A., (1986) Ultrastructural Organisation of spermatocytes in the testes of *Aedes aegypti. Journal of Medical Entomology 23 (4):374-379*

Whitten, M.J., (1970) Genetics of pest in their management in: Concepts of pest management. North carolina state university,

WHO, (1964) Scientific group on genetics and insecticide resistance. World health organisation technical report service **268**:20

Wood, R.J., (1961) Biological and genetical studies on sex ratio in DDT resistant and susceptible strains of *Aedes aegypti* L. Genetica agraria **13**:287-307

Wood, R.J., Newton M.E., (1991) Sex ratio distortion caused by meiotic drive in mosquitoes. The American naturalist **37**:379-391

Wu, C-I., Hammer, M.F., (1991) Molecular evolution of ultraselfish genes of meiotic drive systems, pp. 177-203 in Evolution at the molecular level; Selander, R.K., Clark, A.G., Whittam, T.S., (eds) Sinauer, Sunderland, Mass.

Zimmering, S., Sandler, L., Nicoletti, B., (1970) Mechanisms of meiotic drive. Annual review of genetics **4**:409-436