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Does polyandrous impede mosquito control by autocidal?

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ABSTRACT
Vector mosquito control by releasing genetically altered males has been attempted under the presumption that the females are monandrous. The present observation was through the sex–linked inheritance pattern of eye-colour and the estimation of polyandrous in *in-vitro* mating. A small proportion (18.2%) of the female *Anopheles stephensi* population exhibited polyandrous on examination of 850 F_1 adults when two types of males (white and black eyed) where allowed to mate with homozygous white eyed females. The above results were discussed with relation to the consequences of the polyandrous trait in sterile insect technique, genetic control programmes.

Keywords: Polyandrous, autocidal, *Anopheles stephensi.*

1. Introduction
The Sterile Insect Technique (SIT) is one of the promising biological control of insect pests. In this method an irradiated male sterile individual insects are released in to the dense population. It has successfully controlled the activity of pests *viz*., *Cochliomyia hominivorax* (Screw worm fly) and *Ceratitis capitata* (Medfly) The same procedure of introducing genetically altered sterile males for the control of vector mosquitoes has also been extended. This was under the presumption that the females mate with males only once. Sterile male mosquito compete with wild fertile male mosquito and the female receives the sterile male. Therefore the female mosquito do not have fecundity. Thus, production of eggs is arrested. However these programs have been carried out under the supposition that female mosquitoes are inseminated only once [1].

Gwads and Craig [2], Bullini *et al* [3] Zaloom *et al* [4] and many other earlier workers have observed a stage of polyandry prior to first gonotrophic cycle in a number of mosquito species. The recent molecular studies also confirm the behavior of multiple insemination [5]. On the other hand it has also been shown that polyandry is normally avoided by the action of secretion of the male accessory glands namely the ' matrone' and the mating plug [6]. But the heterozygous hybrid males are said to secrete insufficient matrone to develop refraction and polyandrous is noticed [7]. Misconception and constraints still exist on the control of mosquito by autocidal. Therefore a call for detailed knowledge on genetics and sexual life of mosquito is needed to release the genetically manipulated males for the surest method of eradication by SIT. The present investigation throws light on the mosquito sexual life based on sex-linked inheritance with different theoretical approach.

2. Materials and Methods
The white eyed mutant strain of female *Anopheles stephensi* and wild type black–eyed males of the same species were the materials used in the present study. The white-eyed females was identified while colony of the mosquito collected from the laboratory of NICD, Mettupalayam, India brought to our laboratory for investigation related to the pigment migration in the compound eyes of mosquitoes.

Heterozygous white eyed males (F1) were derived from the white eyed females. The white eyed gene was recessive and the locus was on the X–chromosome. The *Anopheles stephensi* has the heterochromatic sex determination and the Y-Chromosome has no homologous genes to those on the X [8, 9, 10].The mutant white eyed form was tested by salivary gland chromosomal squash. The cytogenetic examination of the mitotic chromosomes (3 pairs) corroborated with the findings of Aslamkhan [11]. The sex chromosome were identical in females (XX) and dimorphic in males. The Y chromosome was slightly small, while the 2

pairs of autosomes were almost identical. As opinioned by the Aslamkhan [11] the inheritance of the recessive, fully penetrate sex-linked mutant, white-eye (w), was due to the homozygous (w/w) in females and male was single gene locus (w) hemizygous.

The larvae were reared in batches of 200 in 30x30cmtrays filled with water, a surface layer of soil with vegetative substance placed at the bottom to promote the growth of microbes also. The temperature (27±2 ^{0}C) and humidity (80%) were maintained. The freshly hatched adults were grasped and classified accordingly to their sex and eye colour phenotypes.

The experimental mating were conducted in cylinders, each containing 50 white-eyed females, 25 white-eyes males and 25 black-eyed wild males. All the mosquitoes were four days old. Males were released after their pivot rotation was over and likely females were allowed to receive their first blood meal prior to mating. Four days after females were separated and placed in an oviposition bowl. The first egg clusters were picked and grown separately. Eye colour of the F_1 progeny was scored in the 4th instar and the sex at adult stage.

3. Results

The results and the statistical analysis are presented in tables 1 & 2.

Table 1: Eye colour phenotypes from 22 egg batches.

S. No	Black eyed Female	White eyed Female	White eyed Male	No. of F_1 examined	Significant
1	21	0	15	36	*
2	18	0	23	41	*
3	8	0	11	19	*
4	29	0	25	54	*
5	9	3	16	28	# (P<0.05)
6	11	0	25	36	*
7	19	0	23	42	*
8	21	0	29	50	*
9	15	9	20	44	# (P<0.05)
10	15	0	22	37	*
11	31	0	25	56	*
12	13	0	18	31	*
13	24	0	16	40	*
14	17	11	24	52	# (1^3<0.05)
15	21	0	27	48	*
16	16	0	23	39	*
17	29	0	18	47	*
18	7	0	6	13	*
19	18	0	13	31	*
20	14	0	11	25	*
21	18	0	15	33	*
22	15	13	20	48	# (P<0.5)

* deviated from the expected ratio.

fit with theoretical explanation, significant at (P<0.05) & (1^3<0.5).

Table 2: Classified phenotypic data in toto.

ITEM	White eyed	Black eyed	Total
Male	425	0	425
Female	36	389	425
Total	461	389	850

If both wild and mutant males are equally participating in insemination and polyandry among female does exist, the expected phenotypes would be 2:1 (White eye: Black eye). It was a surprise to observe that there were equal number of females and males out of 850 insects reared from the egg clusters of 22 experiments sections. Of the 22 groups studied, four batches (5, 9,14 and 22) indicated the presence of white eyed females, while in the rest of colonies there were only white eyed males and black eyed females. As the white- eyed gene is linked to the X- chromosome, males are hemizygous for the locus and so their genotypes can easily be recognized from their phenotypes. The females used in this observation were white-eyed therefore they were homozygous. The outcome of white eyed homozygous female was possible only if she receives the sperm from heterozygous white eyed male. Hence polyandrous quality was noticed in the mating of mosquitoes of units 5,9,14 and 22. The estimated percentage was 18.2 calculated out of 22 sections (Table 1)

The chi-square analysis with Yate's correction factor on each class and on total F_1 adults (850) with expected phenotypic ration of 1:2 (Black: white), showed the present data as statistically insignificant. There were the highest deviations from the presumed values and do not fit with the theoretical means, in lots where the absence of white eyed females were noticed. This would suggest the presence of monandrous trait at majority of instances.

On the other hand, the groups known for the presence of white eyed females (Batch Nos. 5, 9, 14 and 22) also warrant special attention. The application of statistical test yielded the good agreement between the anticipated 1:2 ratios and the observed value (Table 1). This would indicate the occurrence of multiple inseminations in relatively little population (18.2%). In this way, polyandrous condition with the involvement of both types of males could be identified.

4. Discussion

The encounter between the sexes is solely a matter of chance. The mosquito possesses an excellent mechanism that prevents useless ones, and at this point the female holds the dominant role. The present new approach with sex-linked recessive gene for eye colour may be a guide for recognizing the polyandry. The results disclosed that a small population (18.2%) of *Anopheles stephensi* females is polyandrous prior to their gonotrophic Christopher-I cycle. Earlier observations claiming to exhibit polyandry in *Anopheles* species were inadequate in various aspects (Bryan, 1972, Goma, 1963). Besides the recent DNA analysis of inseminated sperms in female mosquitoes, this has also been proved for the existence of polyandrous.

Gomulski [12] employed the same white eyed mutant strain of *Anopheles gambiae* and provided an evidence for polyandrous in 12% of insects studied out of 52 eggs lots unlike to his hypothetical speculation (2:1:1). The results reported here with 1:2 ratio as assumed by different theoretical approximation would be more clear and strong enough of to perceive the monandrous behavior of mating in females and obviously proved the presence of polyandrous traits only among the few.

The cause for the presence of wild males was discussed as mutant hybrid males which have very reduced testes and accessory glands and often lose their vigor [7, 13, 14]. Marchand [15] in his opinion said that competition for female was intense; mating pairs were not disturbed by the unsuccessful male. Mosquitoes in capula leave the swarm to complete the copulation and switch over to “host– seeking’’. Therefore the obstruction while mating, a reason described for polyandrous by Gwadz and Craig [2] is not considered. Despite this, she herself can refract to inseminate again by the action of “matrone” and mechanical block due to the mating plug [3].

Once it was believed that polyandry impeded the successful implementation of mosquito control by releasing genetically manipulated males [13, 16]. (Now it has been ascertained that when the sperm of the altered males contend with that of the wild males in field, in fertilizing eggs, polyandry should not be a subject of question.

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6. References

1. Benedict MQ, Robinson AS. The first release of transgenic mosquitoes an argument for the Sterile Insect technique. Trends in parasitology 2003; 19:349-355.
2. Gwadz RW, Criag GE. Female polygamy due to inadequate semen transfer in *Aedes aegypti* 1970*;* Mosquito News: 30:354-360.
3. Bullini I, Coluzzi M, Bullini BAP. Biochemical variants in a study of multiple inseminations in *Culex pipiens.* Bulletin of Entomological Reasearch 1976; 65:683-685.
4. Zalom FG, Asman SM, Fields JE. Tests for multiple inseminations of females involving irradiated and unirradiated male *Culex* species. Mosquito News 1981; 41,154-156.
5. Tripet F. Toure YT, Dolo G, Lanzaro GC. Frequency of multiple insemination in field collected Anopheles gambiae females revealed by DNA analysis of transferred sperms. American J Tropical Medicine and Hygiene 2003; 68:1-5.
6. Shutt B, Stabies L, Aboagye-Antwi F, Morgan J, Tripet F. Male accessory gland proteins induce female monogamy in anopheline mosquitoes. Med Vet Entomology 2010; 24:91-94.
7. Thailayil J, Magnusson K, Charles H, Godfray J, Crisanti A, Catteruccia F. PNAS Early Edn, 2011; 1-5. Also available in www.pnas.org/cgi/10.1073/pnas.1104738108.
8. Gilchrist BM, Haldane JB. Sex linkage and sex determination in a mosquito, *Culex molestus,* Herditas, 1947; 33:175-190.
9. Manson GF. Genetic studies on mutatations in species A and B of the *Anopheles gambiae* complex. Genetical Research, 1967; 10:205-217.
10. Craig GB, Hickey WA. Genetics of *Aedes argypti* In Wright J.W & Pal, R. Edn, Genetics of Insect vectors of Disesase. Elsevier Amsterdam 1967; 67-131.
11. Aslamkhan M. Sex chromosomes and sex determination in the malaria mosquito *Anopheles stephensi*. Pakistan J zoology 1973; 5(2):123-130.
12. Gomulski L. Polyandry in nulliparous *Anopheles gambiae* Mosquitoes (Diptera: *culicidae)* Bulltein of Entromological Research 1990; 80:393-396.
13. Bryan JH. Further studies on consecutive matings in the *Anopheles gambiae* complex. Nature London 1972; 239,519-520.
14. Klowden M. Sexual receptibility in *Anopheles gambiae* mosquitoes; absence of control by male accessory gland substances J Insect Physiology 2001; 47:661-666.
15. Marchand RP. Field observation on swarming and mating in *Anopheles gambiae* mosquitos in Tanzania Netherlands Journal of zoology 1986; 34:367-387.
16. Schrieber ET, Jones C. Mosquito control hand book. An overview of biological control. Published by University of Florida Department of Medical Entomology USA, 2000; 1-7.