A CYTOLOGICAL STUDY OF SOME PHYSIOLOGICAL MUTANTS OF *CELOSIA ARGENTEA* L. VAR. *PLUMOSA*

BY

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Thesis submitted for the Degree of Master of Science of the University of Sri Jayewardenepura, Sri Lanka

March, 1979

C O N T E N T S

ACKNOWLEDGEMENTS

I express my sincere gratitude to Dr. W.E.Ratnayake, Head, Department of Biological Sciences, Sri Jayewardenepura, University, (Vidyodaya) Nugegoda, for suggesting this problem, for the supervision of this research and for the correction of the thesis and for the kind advice, and constant encouragement given to me at all stages of this project.

I wish to express my gratitude to Professor A.C.J.Weerakoon, (the Head, Department of Biological Sciences, when I started the project) for his constant encouragement, help and advice at all times.

This research work was carried out on a grant (No. 2RG/76/8) from the National Science Council of Sri Lanka, for which I am deeply grateful.

My sincere thanks are also expressed to the following:-

Mrs. N.Jayasekera, Entomologist of the Medical Research Institute, Borella, for the helpful advice given regarding my research work, particularly at the initial stages when I learnt the techniques of handling mosquitoes from her.

Dr. W.A. Samarawickrema, Head Department of Entomology, M.R.I., for the helpful advice given and all the help extended in setting up a mosquito unit at Sri Jayewardenepura University.

Dr. H.G.Nandadasa, for supervision of my research work during Dr. D.E.Ratnayake's absence from the Island from September, 1976 to September, 1977 and for valuable discussions from time to time all along.

Mr. H.Wimalaratne, of the Labour Department for helping me with the photography.

Messrs. H.Dharmasena and M.B. Weerasekera, of the Cancer Institute, Maharagama for helping me in the irradiation of the mosquitoes for certain experiments.

And Mr. Tissa Wickremasinghe, who worked as Laboratory Technician on the project without whom I could not have been able to maintain so many cultures of mosquitoes.

I wish to also thank the members of the Department of Biological Sciences who have helped me in many ways to complete my work and helped to make my stay in the Department a pleasant one.

Finally, I wish to express my deep gratitude to my father for the constant encouragement and sustenance he gave me to pursue my research and for having painstakingly typed the first draft of this thesis, and to my husband, Ranjit, for his kind support and enthusiasm for my work.

A B S T R A C T

1. An attempt was made to obtain morphological mutant of Culex pipiens fatigans Wiedemann, the mosquito vector of filariasis, both from wild type populations and from treated and untreated laboratory inbred colonies. There seems to be some controversy with regard to nomenclature, some authors claiming that the name Culex fatigans is synonymous with many others and that the first named is Culex (Culex) pipiens quinquefasciatus Say (1823), but I have used the name Culex pipiens fatigans throughout my thesis. Spontaneously occuring mutants were isolated from two sets of mosquitoes:

(a) from wild mosquitoes collected from Ratmalana and Nugegoda and then inbreeding them for three generations, and

(b) from two laboratory maintained colonies, one maintained in the lab at room temperature and the other 24°C - 25°C (Air conditioned room).

2. In the case of collections from the wild, a female mosquito that had taken a blood meal was collected fortnightly from a house at about 7 a.m. The F_1 generation was observed from larval to adult stages. Ten F_1 females were intercrossed to F_1 males of the same egg raft in ten separate cages. After mating for seven days, the females were starved and a blood meal was given. Ten egg rafts, one from each female was collected separately and the hatchabilities were recorded. The larval to adult stages were observed. The F_2 adults emerging from each such egg raft were then allowed to intercross and their F_3 progeny was very carefully observed from larval to adult stages for morphological and colour deviants after the hatchabilities had been recorded.

3. From this survey I was able to isolate five phenotypically abnormal types. On testing them, two proved to be definite inheritable mutants, namely, knobbed palp (kp) and unisegmented tarsi (unt). Another mutant hooked legs (hl) arose from the F_2 generation of an outcross of the unt mutant. One deviant proved to be a pheno-copy and the other two phenotypically abnormal types were lost before definite tests could be carried out on them. The phenotypes of these two were, one affecting the legs and the other the antennae. In the former the first tarsal segment of all 3 legs was swollen compared to those of the normal legs. In the latter, both antennae were distinctly shorter than those of the normal mosquito.

4. In the mutant described as knobbed palps (kp) the palps have a knob shaped projection at the junction of the distal two segments. In the normal mosquito the palps are tapering without any visible projections. This knobbed palp mutant was similar to the clubbed palp mutant isolated by Laven in 1959 from Culex pipiens both in phenotypical expression and in genetic characteristics. Although Laven gave the symbol kpg to his mutant I have called it kp. The knobbed palp mutant was found to be due to a recessive, autosomal gene. As it showed expression either in both palps or in one, its expressivity was variable. It showed full penetrance.

5. The second of these mutants, namely, unisegmented tarsi (unt) had only one tarsal segment instead of the usual five. This tarsal segment was shorter than the first tarsal segment of a normal mosquito and it tapered to a point, ending in a single blunt projection. This mutant had not been previously described in the literature, therefore, it has

been given a new name unisegmented tarsi with symbol <u>ust</u>. This mutation was also due to a recessive, autosomal gene and showed variable expressivity and full penetrance.

6. From this <u>ust</u> colony arose another phenotypically distinct tarsal mutant where all the tarsal segments though present had the fourth tarsal segment of the third pair of legs curved giving a hooked appearance to these legs. Analysis showed that this was also due to an autosomal recessive gene. In all the genetic tests for this mutant there was always <u>ust</u> mosquitoes, along with the hooked leg individuals in the F_2 generation and in the backcross. It was suspected that this hooked leg mutation was pseudo - allelic to <u>ust</u> and had arisen by unequal crossing over at the <u>ust</u> locus. But before this idea could be tested, the colony was lost.

7. In addition to the above surveys carried out for the isolation of naturally occuring mutants from the wild, two inbred colonies were maintained in the laboratory, one at room temperature (27°C - 29°C) and the other in an air conditioned room (24°C - 25°C). In the twenty first generation of the colony maintained at room temperature, a mutant was detected which had rough eyes(<u>re</u>), this phenotype had not been described for <u>Culex pipiens</u> before. However, a similar phenotype had been described for <u>Culex tritaeniorhynchus</u> for which the name rough eye and symbol <u>re</u> had been given.

In the mutant I discovered the rough eye was darker and lacked the greenish lustre that is found in the normal eye. Normal mosquitoes have eyes that are greenish tinged dark brown in colour. In addition to this colour difference from the normal, the corneal surface of

the eye, instead of showing the smooth and clear separation of regularly arranged ommatidia, appeared fused in patches and, therefore, gave a black and rough appearance to the surface of the eye. These patches varied in size. Genetic tests did not give a clear 3:1 ratio or 1:1 ratio expected for an intercross and a backcross respectively for a recessive gene. The observed values departed significantly from the expected. It is hypothesised, however, that this mutant gene is recessive and probably linked to the sex gene and that the departure from expected ratios is due to (a) mortality of the homozygous mosquitoes and (b) incomplete penetrance.

8. Recovery of heterozygously existing mutations from wild populations enabled us to detect frequencies in such populations for Culex pipiens fatigans in and around Colombo, which have been sampled by me. I have obtained a frequency of 0.06 mutation/individual. If the two aberrant mutations that were lost before testing were considered as true mutants and if they are also included then the mutation frequency becomes 0.12 mutation/mosquito. Then the frequency obtained for wild population from Sri Lanka approximates that of 0.16 mutation/mosquito obtained by VandeHey in 1969 from Germany.

Similar frequencies for other mosquitoes, particularly Aedes aegypti, are much higher. It is hypothised that this difference in mutations/individual between Culex pipiens and Aedes aegypti is due to differences in their egg laying habit, Culex pipiens laying one single egg raft of 100 - 200 eggs at a time whilst Aedes lays a few eggs separately at a time.

9. Irradiation (X-rays) and chemical (Ethy methane Sulphonate, fluoro uracil and thiotepa) treatment were also used to obtain mutants. No morphological or colour mutants were obtained. This may be because the induction of visible mutations is generally very rare in most organisms. The few that had been recorded elsewhere as arising from treatment may have actually been pre-existing or spontaneously arising ones.

10. This project was started with the hope of building up mutant stocks of the mosquito Culex pipiens fatigans, Wiedemann the vector of human filariasis in Sri Lanka, from which special tester stocks could be built up, for the detection of translocations and lethal mutations. These translocations were to have been used eventually in genetic control programmes of the mosquito. However, in April 1978 when I had completed only the experiments reported in this thesis, all my mosquito colonies died out for some reason, may be the climatic conditions prevailing at that time or some other cause.

1. INTRODUCTION

1.1 Mosquitoes and diseases

Man's worst natural enemy is still the mosquito being the most important vector of human and animal diseases. Mosquitoes carry diseases which cause tremendous economic loss and human misery. In addition they are conspicuous nuisance pests as well.

Some of the more important diseases that are transmitted by mosquitoes are yellow fever, malaria, filariasis, encephalitis and dengue. These diseases afflict people of both developing and developed countries.

The first concrete evidence that mosquitoes are involved in the transmission of disease was presented by Manson in 1878 where he showed that the nematode Filaria sanguinis hominis (now known as Wuchereria bancrofti) underwent development in a 'brown mosquito' (Culex fatigans). In 1898 the discovery of the mosquito cycle of malaria by Ross revolutionised the outlook on mosquitoes and other biting insects and practically founded modern tropical medicine. Reed and his collaborators in 1901 discovered that yellow fever was transmitted by the mosquito Aedes aegypti.

In Sri Lanka the two common mosquito borne diseases are malaria and filariasis. Abdul Cader (1962) referring to the history of filariasis in Sri Lanka has stated that the earliest reference to the prevalence of the disease was made by John Davy in 1821. Reference

to the incidence of the disease was also made in the 1879 Administration Report of the Principal Civil Medical Officer and Inspector General of Prisons. The incidence and distribution of filariasis in the country known only in 1939 with the publication of the results of large scale survey by Dassanayake.

The vector of filariasis in Sri Lanka has been known as Culex pipiens fatigans, Wiedemann for a long time. This vector is found in all urban areas of Sri Lanka, yet, human filariasis has been shown to be endemic only on the south western coastal belt extending from the north of Negombo to the east of Matara, urban filariasis being caused by the nematode Wuchereria bancrofti, while rural filariasis caused by Brugia malayi is transmitted by the Mansonia mosquito. This form rural is now supposed to be eliminated from Sri Lanka.

Malaria has been prevalent in Sri Lanka for many centuries. The earliest record of its existence is seen in a map published in 1638 by the Dutch. Twenty species of Anopheline mosquitoes had been incriminated as the vector in the world. James & Gunasekera in 1913 have incriminated Anopheles culicifacies as the vector responsible for malaria in Sri Lanka. Nearly 4/5 of the country was subjected to endemic malaria and a large portion of the thickly populated balance area had severe regional epidemics periodically (Visvalingam, 1961).

Another of the mosquito borne diseases in Sri Lanka is Dengue which has been known for some time to have been endemic in the Island. The first recorded epidemic of the disease, however occurred as recently as 1965 in the south western coastal zone.

Japanese encephalitis virus was isolated for the first time in Sri Lanka from a post mortem on a dead child near Colombo in 1969 (Hermon and Anandarajah, 1974). The disease is not common in Sri Lanka. The recognised vectors of Japanese encephalitis in South Asian regions are Culex gelidus and Culex tritaeniorhynchus which are present and widely distributed in this country.

1.2 The Genetics of Aedes, Anopheles and Culex tritaeniorhynchus

Ganetic studies of mosquito vectors was started in earnest only very recently when it was shown by Serebrovsky in 1940 that certain genetic phenomena like translocations could be used in their control. However, formal genetic studies had been initiated about twenty years earlier with the rediscovery of Mendel's Laws in 1900 by Correns, Tschermak and de Vries and when the applicability of Mendel's Laws were being tried out on various organisms.

A large number of morphological, physiological and biochemical mutants in mosquitoes have been recorded since then. A fairly detailed genetic study on mosquitoes started with the discovery of white eye by Gilchrist and Haldane in 1947.

I shall review only the mutants of Aedes, Culex tritaeniorhynchus and Anopheles in this section. The mutants of Culex pipiens will be discussed in a later section.

White eye appears to be a common eye mutant in mosquitoes and in fact in all insects. Baker in 1969 has referred to the fact that white eye mutants have been reported in Anopheles gambiae, Anopheles quadrimaculatus by Kitzmiller and Mason in 1967, and in Culex tritaeniorhynchus by Baker in 1969. White eye has also been reported in Aedes aegypti (Bhalla, 1968)and in Aedes cooki (Wade, 1977). In all these species this mutant appears to be sex linked and recessive. In Culex tritaeniorhynchus white eye females were found to be generally sterile. This mutation expresses itself in egg, larval, pupal and adult stages.

A probable allele of white eye, rose eye (w^{re}) has been described in Culex tritaeniorhynchus (Baker and Sakai, 1973a). Rose is a sex linked recessive mutant and the results of crosses suggest that white eye (w) and rose eye (w^{re}) are co-dominant alleles and the heterozygote (pale rose) can be distinguished from both homozygotes that is from white eye and dark rose. These white eye alleles have also shown very interesting physiological effects. The rose eye allele was found to be temperature sensitive (Baker and Sakai,1974a). This allele is dominant to white eye at 26°C and recessive to wild type (brownish black) at all temperatures (24°C - 34°C). Both white (w) and wild type (w^+) eye colours are temperature insensitive. When pre adult development of the rose eye mutant occurred at 20°C - 30°C the mutant eye colour is rose. At 32°C, the adult eye was completely white. If development occurred at 26°C the adult eye was a dark rose. When the temperature was raised to 34°C, the eye was completely white and if lowered to 24°C the colour was dark rose. Temperatures intermediate between these two extremes gave intermediate eye colours, from white, pale pink, rose to dark red.

Another rose eye mutant in Culex tritaeniorhynchus (w^{re2}) which is an allele of white eye was produced in a laboratory colony originally treated with ethyl methane sulphonate (EMS), but this unlike the former was temperature insensitive.

In Anopheles culicifacies Giles, rose (re) in which both the ocellus and compound eye are a bright rose was discovered following treatment with ethyl methane sulphonate (Sakai, Ainsley and Baker, 1977). In the female this locus is sex linked and recessive, while males are hemizygous for this locus.

Originating from the white eye stock in Culex tritaeniorhynchus a spontaneously occuring mutant, red spotted eye (Rs) was described (Rabani and Baker, 1970). This probably represents the first described variegated eye mutant under the genetic control of an autosomal co-dominant gene that can only be expressed under the condition of homozygosity at the sex linked locus (white eye). The normal allele at the white eye locus is epistatic to the red spotted eye gene. Although white eye itself is a sex linked mutant, linkage tests show that Rs is an autosomal mutant placed in linkage group II.

Two other eye mutants that have been recorded so far are brown (bw) and abnormal eye (ae) in Culex tritaeniorhynchus (Sakai, Baker and Iqbal, 1976).

Abnormal eye (ae) is characterised by a slight gap between the eyes on the ventral surface of the head, the size of the eye being slightly reduced and the outline rounded.

Brown eye (bw) as the name indicates produced brown coloured eyes. Both mutants are recessive and autosomal and are under the control of loci in linkage group III. A third new phenotype 'Yellow orange eye' is exhibited by Culex tritaeniorhynchus for both brown and rose ($bw/bw;w^{re}/w^{re}$). If the rose allele associated here is temperature sensitive (w^{re1}) the eyes are white at 32°C and pale orange at 26°C. However if the rose allele is insensitive (w^{re2}) the eyes are pale orange at both temperatures.

Some additional eye mutations have been reviewed by Kitzmiller (1976) and are as follows:-

Landani et al in 1970 has recorded red eye mutant (re) in Anopheles atroparvus which is due to an autosomal and recessive gene.

Two genes affecting eye colour, red and rust, have been described in Aedes aegypti by McClleland in 1966. Both these mutants are recessive and partially sex linked and constant in expression showing complete penetrance. In the salt marsh mosquito Aedes taeniorhynchus, a bleached eye mutant was isolated by O' Meara in 1975 which is autosomal and recessive, but showing a pleiotropic lethal effect dependent upon the genotype of the mother.

Craig and Hickey in 1967 has referred to the olive eye mutant in Aedes aegypti in which the eyes are reddish green to brown in colour.