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Four new morphological mutants of *Culex pipiens fatigans*, Wiedemann (*Culex quinquefasciatus*, Say) from Sri Lanka

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Abstract : Nineteen engorged females of *Culex pipiens fatigans*, Wiedemann, from Nugegoda and Ratmalana, Sri Lanka, were examined for mutant progeny. Two mutants, unisegmented tarsi (*ust*) and knobbed palpi (*kp*) were isolated from them and were tested genetically. Two other aberrant mosquitoes, one with swollen tarsus and the other with short antennae respectively, were lost before tests could be carried out on them. Another proved to be a definite phenocopy.

From these mutants it has been calculated that the heterozygous mutant load in *C. fatigans* in the wild for Sri Lanka is 0.06 mutations/mosquito (or 0.12 mutations/mosquito if the two lost mutants are also considered). This value is seen to be close to the value obtained for *C. pipiens* in Germany and is much lower than the values obtained for *Aedes aegypti*.

Another mutant, hooked legs (*hl*) arose from a cross of the *ust* mutant and is thought to be a pseudoallele of *ust*.

From a laboratory bred colony of *C. fatigans*, an eye mutant rough eye (*re*) was also obtained.

Mutants unisegmented tarsi (*ust*), hooked legs (*hl*) and rough eye (*re*) are described for the first time in *C. fatigans*. Unisegmented tarsi (*ust*) has not been recorded for any mosquito hitherto. Knobbed palpi (*kp*) has been recorded for *C. pipiens* but not for *C. fatigans*.

All four mutants are due to autosomal and recessive genes (excepting perhaps rough eye) whose expressivity is variable.

1. Introduction

Culex fatigans, the common house mosquito, is the vector of the urban filaria nematode (*Wuchereria bancrofti*) and is found in all urban areas of Sri Lanka. This mosquito is, therefore of considerable medical importance. Genetic studies on *Culex* started with the discovery of the white eye mutant by Gilchrist and Haldane in 1947. Of late, due to interest in genetic methods of control, the formal genetics of the mosquito is being studied earnestly and many mutants have been isolated, identified and analysed.[3,8,9]

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The present paper reports the isolation and study of four morphological mutants of *C. fatigans* obtained from natural populations from two locations close to Colombo, Sri Lanka. From these studies, it has also been possible to estimate the frequency of heterozygously existing mutants for mosquitoes in Sri Lanka.

2. Materials and Methods

Spontaneously occurring mutants were isolated from two sets of mosquitoes : (1) from wild mosquitoes collected from various locations in Ratmalana and Nugegoda, and then inbreeding them for three generations and (11) from a colony maintained in the laboratory at room temperature.

(I) Two engorged females *C. fatigans* mosquitoes were collected fortnightly from Ratmalana and Nugegoda between 7 a.m. and 8 a.m. After the identification had been checked, these mosquitoes were caged. The cage was 6″ × 6″ × 6″, where the top and one side were of wire mesh, two sides were of wood, the other was of glass and the remaining side had the sleeve. The females were kept without sugar solution for 48 to 72 hours until the blood meal had digested.

The females were then transfered for egg laying, each into a plastic cup (2½″ diameter × 2″ height) containing a strained solution (about 40 ml) of straw infusion. The mouths of these cups were covered by a piece of muslin cloth held in place by a rubber band. The egg rafts that were laid were transfered to an enamel basin (3 diameter) containing 450 to 500 ml tap water. F_1 larvae that hatched out in about 24 hours were fed daily on Bemax and dried yeast powder (about 0.03 gm). The adult mosquitoes were fed on 10% sugar solution. 5 ml of sugar solution were kept in vials (3″ height × 1″ diameter) with wicks made from toilet tissues that were replaced every 3 days. Ten F_1 females were allowed to mate for 7 days and then starved for 24 hours after which chick blood meal was given. The females were then starved for 48 to 72 hours, isolated individually for egg laying in plastic cups containing straw infusion. The hatchabilities of the eggs in each raft were recorded. The F_2 adults emerging from each raft were observed and then allowed to intercross and their F_3 progeny was very carefully observed from larval to adult stages for morphological and colour deviants.

Once a suspected mutant type was observed attempts were made to obtain a pure breeding colony from it. From these pure colonies that were ultimately obtained, genetic crosses were made to study the mode of inheritance of the mutant gene.

(II) The laboratory colony maintained at room temperature was started from a single egg raft collected from Ratmalana in 1976.

In this colony the adults were intercrossed in mass in $1' \times 1' \times 1'$ cages. The larvae of ten egg rafts from each colony were reared together in a trough (1′ in diameter) and were provided with Bemax and dried yeast as food. The same feeding and breeding protocol as described above were followed. In each generation, larvae, pupae and adults were screened for mutants.

(III) Genetic Crosses.

The following genetic crosses were carried out once a pure breeding mutant colony was obtained.

(*a*) Mutant ♀♀ x wild ♂♂ P_1
(*b*) Mutant ♂♂ x wild ♀♀ P_2 (reciprocal of P_1)
(*c*) F_1 intercross of P_1
(*d*) F_1 intercross of P_2
(e) F_1 backcross of P_1
(f) F_1 backcross of P_2

3. Results

3.1 The Mutants

Four mutants were isolated and have been given the names (I) unisegmented tarsi (*ust*), (II) hooked legs (*hl*), (III) knobbed palps (*kp*) and (IV) rough eyes (*re*).

Actually, we were able to isolate 5 phenotypically abnormal types from the wild population. But on testing them, two proved to be definite inheritable mutants, namely *ust* and *kp* while one was a definite phenocopy. Two were lost before they could be made into pure breeding colonies, but we suspect that they were true mutants. The mutant *hl* arose from the F_2 generation of an outcross of the *ust* mutant.

In the twenty first generation of the inbred laboratory colony maintained at room temperature, the mutant named rough eye (*re*) was detected, which obviously must have arisen as a mutation.

(1) *Unisegmented tarsi* (*ust*) (See Plate 1)

In this mutant the last two pairs of legs are short due to the presence of only one tarsal segment in each leg which is comparatively shorter than the first tarsal segment of a normal leg. The tarsus of a normal leg has five tarsal segments and the proximal tarsal segment is of approximate length 2mm and the distal one ends in two claws, whereas the legs of the *ust* mutant have only one tarsal segment (approximate length 1 mm) each which ends in a blunt projection. It was observed that in the pure breeding colony of *ust* and in all crosses made from it a large number of adults (about 10% to 20%) drowned while emerging from their pupal cases with their legs

still stuck to the cases. An attempt was made to dissect such drowned adults out of the pupal cases to observe the nature of their legs. Out of 100 such attempts only in 15 cases could the entire mosquito be dissected out successfully with their short legs fully intact, and of these 15, the phenotype was always found to be *ust*; not one was found to be normal. All drowned adults in crosses of this strain were, therefore, considered to be *ust*. In some *ust* mosquitoes, only the last pair of legs showed the abnormality. This could imply that the *ust* gene was of variable expressivity.

(*II*) *hooked legs* (*hl*) (See Plate 2)

This mutant was isolated from the F_2 egg raft of an outcross of the mutant *ust*. In this mutant all the tarsal segments are present as in a normal leg but the last pair of legs are hooked in appearance. It is the fourth segment of the tarsus which is curved backwards. In the normal leg the tarsus is straight. It was observed that in some mutants only one leg of the last pair of legs was hooked. Therefore expressivity is seen to be variable.

(*III*) *knobbed palps* (*kp*) (See Plate 3)

The palps of this mutant 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. Here, too, in some mutants only one palp was knobbed.

(*IV*) *rough eyes* (*re*)

The rough eye was darker and lacked the greenish lustre that is found in the normal eye. Normal mosquitoes have eyes that are dark brown in colour but showing also a greenish tinge. 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 as found in the normal eye, appeared fused in patches and therefore, gave a black and rough appearance to the eye.

3.2 Genetics tests

Genetic crosses described in the section on Materials and Methods (2) were conducted on each of the mutants mentioned above.

A pure colony was not obtained for *kp*, but in attempts to obtain such a pure breeding colony, records of the matings were kept and from those results the mode of inheritance was determined. The results of the genetic crosses of the four mutants are presented in Tables I, II, III, and IV.

PLATE 1. The mutant phenotype of *unisegmented tarsi* (*ust*) of *Culex fatigans*

PLATE 2. *Hooked legs* (*hl*) phenotype of *Culex fatigans*

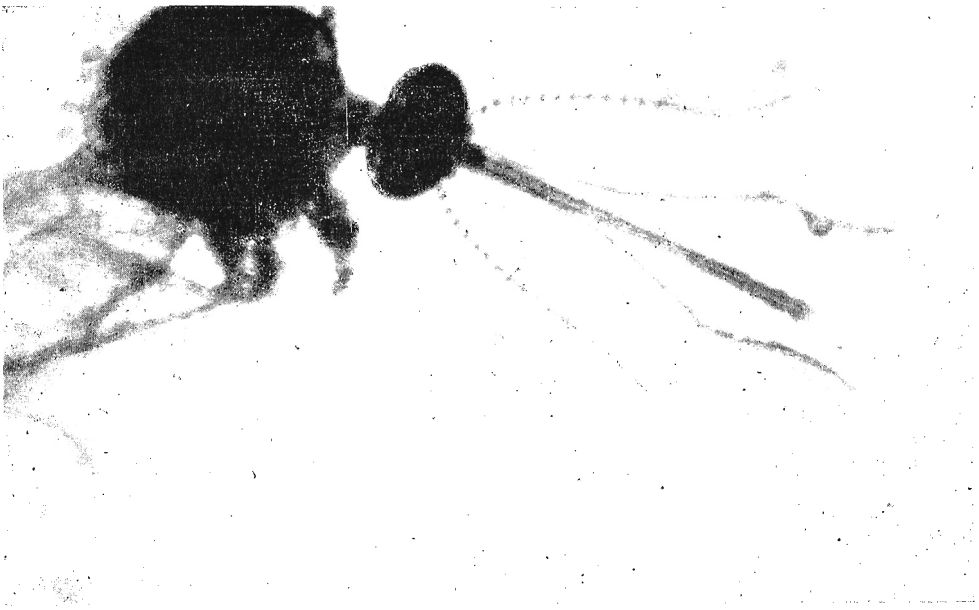

PLATE 3. The *knobbed palp* (*kp*) mutant of *Culex pipiens fatigans*

The other phenotypically abnormal types discovered were lost before definite tests could be carried out on them, but were seen to be inherited into a few generations suggesting they were true mutants. The phenotypes of these too were, one affecting the legs and the other affecting the antennae. In the former, the first tarsal segment of all legs were swollen (swollen tarsus) compared to those of the normal legs. In the latter, both antennae were distinctly shorter (short antennae).

(*I*) *Unisegmented tarsus—ust* (See Table 1)

In the parental outcrosses all the resulting F_1 adults have normal legs. This indicates that the mutant allele is recessive to the normal allele.

The reciprocal intercrosses fit a 3:1 ratio expected for the segregation of a single pair of recessive genes.

However in cross No. 3 a heterogeneity χ^2 test for wild type *vs.* mutant in the adults emerging from individual egg rafts showed that there was a considerable variability between the 13 egg rafts. It appears that two egg rafts have contributed to the high heterogeneity χ^2 values obtained. If these two aberrant egg rafts are not considered then the results of this cross fits a 3:1 ratio. A heterogeneity χ^2 test applied to the F_2 results of cross No. 4 showed that there was no variability between the 9 egg rafts.

The results of the backcrosses showed a clear segregation of wild and *ust* gene in a 1:1 ratio that is expected for a recessive gene. A heterogeneity χ^2 test showed that there was no variability between the egg rafts.

In all the above experiments, the drowned adults have been considered as *ust* (See 3:A1). The phenotypic character sometimes appeared only in one leg.

From the same results it was also possible to test whether the *ust* gene was linked to the sex genes *m* or *M*. In the intercrosses ($+^{ust}$/ust ♂♂ × $+^{ust}$/ust ♀♀ and reciprocal cross), a 3: 1: 3: 1 ratio and for backcrosses a 1: 1: 1: 1 ratio is expected. For $+^{ust}$ ♂♂ : ust ♂♂ : $+^{ust}$ ♀♀ : ust ♀♀, if the mutant gene is unlinked to sex. Significant deviation from this would indicate linkage. Of course, if the genes are linked but are over 50 crossover units apart then they will appear to be unlinked and will assort independently and then too a 3: 1: 3: 1 or a 1: 1: 1: 1 ratio could be obtained. With regard to the intercross as a 3 : 1 test for $+^{ust}$ for both males and females together has already shown independent segregation, only a 1 : 1 ratio of *ust* ♂♂ and *ust* ♀♀ need be tested. Similarly for the backcross only a 1 : 1 test for *ust* ♂♂ and *ust* ♀♀ have to be tested.

The χ^2 values for such tests are given in column 11 of Table 1. It is seen that (except for cross 3) the *ust* gene and sex gene are assorting independently. This would indicate that the *ust* gene is unlinked to sex (or even if linked is over 50 crossover units apart).

TABLE 1. Results of the Genetic Crosses of the *ust* Mutant.

1	2	3	4	5	6	7	8	9	10	11
Cross No.	Experimental Cross	Total No. of Eggs	%Hatchability	+ ust ♂	+ ust ♀	ust ♂	ust ♀	χ^2 (1) for Total No. of Adults	Heterogeneity χ^2	χ^2 for Sex
1.	Parental Cross P_1 ust ♀♀ × + ust ♂♂	1091	94.4	337	297	0	0	—	—	—
2.	Parental Cross P_2 ust ♂♂ × + ust ♀♀	1720	91.3	751	690	0	0	—	—	—
3.	F_2 Results of P_1	1922	91.5	310	324	85	143	For 3:1 >0.96 P>0.2	37.65 df<=12 P<0.001	For 1:1 <14.75 P<0.01
4.	F_2 Results of P_2	1644	96.2	221	224	70	70	For 3:1 >0.36 P>0.5	3.36 df>=8 P>0.9	For 1:1 0
5.	Backcross of P_1&P_2	1176	69.0	171	195	150	185	For 1:1 >1.37 P>0.2	6.01 df>=8 P>0.5	For 1:1 3.65 >df=1 P>0.05

The variation in the cross No. 3 may be due to the presence of the two aberrant egg rafts in that cross.

(*II*) *Hooked legs—hl* (See Table 2)

The F_1 adults all have normal legs phenotypically, indicating that the mutant allele is recessive to the normal allele.

The F_2 results show that there are three types of adults, namely wild (+*hl*) type, hooked legs (*hl*) type, and unisegmented type (*ust*). In the intercross and the backcross, the *hl* and *ust* type were considered together as one group when trying to fit the results to an expected model. In both intercrosses, the results fit a 3 : 1 ratio expected for a recessive gene, although the backcrosses do not fit a 1 : 1 ratio. There was a very high mortality of the larvae in this backcross and the anomalous results cannot be satisfactorily explained. As the colony was lost soon after this test, further tests could not be carried out.

(*III*) *Knobbed palps—kp* (See Table 3)

These results were actually obtained when *kp* was intercrossed to obtain a pure colony of its mutant. Though several attempts were made to obtain a pure breeding mutant colony, they all proved abortive.

In the parental intercross it was observed that all the F_1 mosquitoes showed normal palps, therefore it can be concluded that the *kp* is a recessively inherited character.

The results of the F_2 generation of the genetic crosses for total number of mosquitoes (total of males) fits a 3 : 1 ratio which is expected for the segregation of single pair of genes. A heterogeneity χ^2 tests for adults emerging from individual egg rafts showed that there was no variability between the egg rafts.

The results of the backcrosses do not show a clear segregation of wild and *kp* as expected.

A heterogeneity χ^2 test for adults emerging from individual egg rafts showed that there was no variability between the egg rafts considered. It was also observed that in all these crosses in some males both the palps were knobbed while in others only one palp was knobbed. Considering the results of all these tests it can be concluded that *kp* is a recessive, sex limited autosomal gene with variable expressivity, but having full penetrance.

(*IV*) *Rough eyes—re* (See Table 4)

The F_1 results of the reciprocal parental outcrosses (Table 4) show that all the adults are normal phenotypically. This indicates that *re* is a recessively inherited character.

TABLE 2, Results of the Genetic Crosses of the *hl* Mutant.

1	2	3	4	5	6	7	8	9	10	11
Cross No.	Experimental Cross	Total No. of Eggs	% Hatchability	$+^{hl}$ ♂	$+^{hl}$ ♀	hl ♂	hl ♀	ust ♂	ust ♀	$\chi^2_{(1)}$ for Total No. of Adults
1.	Parental Cross P_1 hl ♂♂ × $+^{hl}$ ♀♀	1297	98.9	367	369	0	0	0	0	—
2.	Parental Cross P_2 hl ♀♀ × $+^{hl}$ ♂♂	806	98.7	260	268	0	0	0	0	—
3.	F_2 Results of P_1	1250	99.5	282	232	47	42	29	20	For 3:1 5.1 df=1 P>0.02
4.	F_2 Results of P_2	2645	99.5	103	175	27	25	25	11	For 3:1 0.19 df=1 P>0.5
5.	Backcross of P_1&P_2	481	63.8	32	31	8	8	3	8	For 1:1 14.4 df=1 P>0.001

TABLE 3. Results of the Genetic Crosses of the *kp* Mutant.

1	2	3	4	5	6	7	8	9
Cross No.	Experimental Cross	Total No. of Eggs	%Hatchability	$+^{kp}$ ♂	One Palp kp ♂	Both Palps kp ♂	$\chi^2_{(1)}$ for Total No. of Adults	Heterogeneity χ^2 for Total No. of Adults
1.	Parental Cross P_1 kp ♂♂ × $+^{kp}$ ♀♀	2071	98.6	672	0	0	—	—
2	F_2 Results of P_1	1345	98.6	142	25	17	For 3:1 0.45 P>0.5	7.52 df=8 P>0.2
3.	Backcross of F_1	1457	96.4	225	112	52	For 1:1 9.53 P>0.001	13.87 df=9 P>0.1

TABLE 4. Results of the Genetic Crosses of the *re* Mutant

1	2	3	4	5	6	7	8	9	10
Cross No.	Experimental Cross	Total No. of Eggs	%Hatchability	$+^{re}$ ♂	$+^{re}$ ♀	re ♂	re ♀	$\chi^2_{(1)}$ for Total No. of Adults	Heterogeneity χ^2 for Total No. of Adults
1.	Parental Cross P_1 re ♂♂ × $+^{re}$ ♀♀	1495	67.2	213	252	0	0	—	—
2.	Parental Cross P_2 re ♀♀ × + ^{re} ♂♂	280	84.3	46	84	0	0	—	—
3.	F_2 Results of P_1	2663	77.7	418	404	103	77	For 3:1 26.3 P<0.001	27.45 df=16 P>0.02
4.	F_2 Results of P_2	1692	88.1	159	173	19	16	For 3:1 47.21 P<0.001	15.52 df=8 P>0.02
5.	Backross of P_1 & P_2	2920	69.7	247	280	95	103	For 1:1 149.49 P<0.001	22.46 df=17 P>0.10

The results of the F_2 progeny of the intercrosses do not fit a 3 : 1 ratio expected for the segregation of a single pair of recessive genes. The heterogeneity χ^2 test also showed that there is a variability between the egg rafts concerned.

The results of the backcrosses also do not fit the 1 : 1 ratio expected for a recessive gene. As the fit for 3 : 1 (intercross) and 1 : 1 (backcross) ratio was not clearly significant, the 1 : 1 *re*♂ : *re*♀ test for both intercross and backcross were not considered. From the above results it is difficult to make any definite conclusions with regard to this mutant gene.

4. Discussion

4.1 The Mutants

Of the four mutants described only knobbed palp (*kp*) has been previously described for *C. pipiens* complex before and the rest are being described for the first time.

Laven[9] had given a description of the mutants which he had isolated from *C. pipiens* in 1955 in which the palps were affected. These mutants show a similar phenotype to the knobbed palp mutant we have isolated. Laven described clubbed palps in *C. pipiens* as follows: "The expression was symmetrical and the distal segment of the palps of the male was shortened and contracted to club-like or hooked-shaped form. The females showed no expression of the gene. In all cases, the expression was symmetrical in both palps while it rarely affected one palp only." Laven has concluded that "This mutant was due to a recessive, autosomal gene with good penetrance and with variable expressivity." There seems to be a doubt as to whether this mutant was induced or had arisen spontaneously.

In 1958, a similar mutant was isolated by Kitzmiller after irradiation. He found that something more than a simple recessive gene was involved. Also that tests for allelism gave very erratic expression of the two genes. It is possible that the two mutants obtained by Laven and Kitzmiller were due to two different sets of genes but with identical phenotypes (mimic genes).

A similar phenotype but with asymmetrical clubbing has been recorded again by Laven in 1957. The clubbing is more pronounced in one palp than in the other. Also the last palpal joint was often only bent outwards. This phenotype was due to a dominant sex-linked gene, without full penetrance and of variable expression and the sisters of the affected males did not transmit the trait.

Kitzmiller's mutant with asymmetrical palps did not behave in the same way. It was neither clearly dominant nor typically recessive. Whether it was sex-linked has not been clarified and there is a possibility that this and other similar phenotypes represent chromosomal changes (translocations) which could explain sex-linked inheritance on the one hand and autosomal inheritance on the other.

The mutant, knobbed palps (*kp*), that we found had a similar phenotype to that discovered by Laven in 1955. The palp at the junction of the distal segments of the palps had a swelling which was knob-shaped. Attempts to obtain a true breeding colony with our mutant were unsuccessful. When the knobbed palp males were outcrossed to wild type females, the F_1 progeny were all of the wild type. The F_1 intercross gave 3 : 1 ratio (wild type : mutant) expected for a recessive mutation. The backcross of the F_1 females crossed to knobbed palp males did not give a clear-cut 1 : 1 ratio as expected. The knobbed palps males were found to be of two types. In some, the expression was symmetrical affecting both palps equally while in others it was asymmetrical, only one palp being knobbed and the other normal with a very small swelling. In the symmetrical clubbed palp described by Laven in 1955, the expression is symmetrical in both palps and rarely only one palp is affected. It is also recessive and autosomal.

Similar types of mutants have also been described in mosquitoes other than *Culex pipiens*, by Bat-Miriam and Craig (Jnr.)[4], Craig and Hickey[6] and Kitzmiller.[8]

So it seems that mutations affecting the palps in mosquitoes are fairly common in nature. The mutant which we have isolated from *C. fatigans* seems more like the first mutant obtained by Laven in 1955 for *C. pipiens*.

Unisegmented tarsi, the second of the mutants isolated by us, is a spontaneously occurring mutant isolated in the F_2 progeny of a fertilised female caught at Ratmalana. This was found to be an autosomal, recessive mutation, with full expressivity but with variable penetrance.

Hooked leg, the third mutant isolated by us came from the F_2 progeny of an unisegmented tarsi female. This was found to be autosomal, recessive and affecting both males and females. The fourth segment of the tarsus being curved by about 60°, gave the appearance of a hook.

Laven[9] had referred to the work of Kitzmiller in 1958, who by irradiation had obtained a phenotype with curled hind tarsi in *C. pipiens*, and according to him this was not inheritable and therefore was most likely a phenocopy. Craig (Jnr.) and Hickey[6], had recorded a similar mutant in *Aedes aegypti*, called "tarsi hooked" discovered by McClelland in 1962. In this mutant, the segments 4 and 5 of the metathoracic tarsi are sharply recurved dorsally, forming a distinct hook. In the pupa the legs are bent in a gentle curve. A few adults die during eclosion because they cannot extract their legs. Expression in adults is somewhat variable, in that the hook is not always complete. Sometimes the tarsal segments 4 and 5 break off and remain in the pupal case.

In *Culex tritaeniorhynchus* another mutant named 'curved legs' had been isolated[1] in which the shape of the metathoracic leg tarsi are modified, segments 4 and 5 are sharply recurved forming a distinct hook. The expressivity is variable, ranging from a 290° turn to only a slight bend in one tarsal segment. In addition, tarsal segments are frequently broken and missing. This was found to be inherited as an autosomal recessive.

Our mutant 'hooked legs' gave a clear cut phenotype and was quite distinct from 'unisegmented tarsi'. As *hl* arose from a colony of *ust*, obviously there was some relationship between the two which we reckoned to be genetic. Before tests for allelism (pseudo-allelism) could be completed, however, all our colonies of mosquitoes suddenly died out.

Rough eye, the fourth mutant in this series of mutants described by us, was discovered from a laboratory colony of *C. pipiens fatigans* maintained at room temperature. The F_1 results of the parental crosses P_1 and P_2 indicate that the mutant gene is recessive because all the F_1 progeny were of the wild type. But the F_2 results and the results of the backcrosses do not agree with 3 : 1 ratio and 1 : 1 ratio expected for a recessive mutation. The only way to explain the departure from expected ratio is to assume that the mutation shows great variability in both penetrance and expressivity, for as already referred to the roughness of the eye is very patchy and was difficult to identify unambiguously.

Baker and Sakai[2] have referred to a similar type of mutant, rough eye in *C. tritaeniorhynchus* in which the pupa and adults have rough eyes with facets irregular in size and arrangement. The penetrance here is complete, while expressivity is variable.

4.2 Heterozygous frequency of mutant genes

A number of workers have tried to estimate the frequency of mutations occuring naturally in the heterozygous condition in populations of various species of mosquitoes.

The incidence of genetic mutations in *C. pipiens* populations had been studied by VandeHey.[13] The analysis indicated that the sample of 13 inseminated females from Bodenheim, Germany, had a mutation frequency of 0.32 per pair or 0.16 per mosquito.

We collected engorged females of *C. pipiens fatigans* one at a time every fortnight, from two locations near Sri Jayewardenepura University Campus, i.e., Nugegoda and Ratmalana. F_1 progeny were obtained from each female and 10 virgin females from each such F_1 females were intercrossed separately to 10 males from the same breed and the F_2 progeny (i.e., F_2 progeny from 10 egg rafts from each parental female) were screened for mutants. From the 11,113 F_2 adults examined

from 19 females, we were able to isolate only 6 different abnormalities. Of these 3 were found to be truly genetic, while two others were lost before definite tests could be carried out on them and one proved definitely to be a phenocopy, as it was not inherited to subsequent generations. The 3 mutants isolated were knobbed palps (*kp*), unisegmented tarsi (*ust*) and hooked legs (*hl*). The *kp* and *ust* mutants were isolated directly from the F_2 progeny of two wild females, while the 'hooked leg' mutant was isolated from the F_2 progeny of an outcross of the *ust* mutant mosquitoes. Therefore only two mutants were really isolated from the wild.

If the two that were lost before testing are also considered as true mutants, then altogether 4 mutants were isolated. We shall consider both cases, that of 2 mutants and 4 mutants respectively, in our estimation of mutation frequencies in the population. It is seen that the sample examined had a mutation frequency of 0.06 mutation/individual if 2 mutants are considered or 0.12 mutation/individual if 4 mutants are considered. This value was calculated from the formula $E\text{-}R/1—(\frac{3}{4})^n$ given by Craig and Hickey[5] (modified from Spencer, 1947), where E is the number of mutants estimated to be present in a given pair tested, R is the number of mutants recovered in the F_2 and *n* the number of F_1 pair matings that produce offspring. The summation of E values for each pair tested gives the total number estimated for the population. No mutants were recovered in the F_3 and F_4 progenies. Compared to those obtained by VandeHey (0.16 mutation/mosquito) the frequency we observed (0.06 mutation/individual) was very low. The explanation we have to offer for this difference is that it may be (a) due to differences in screening methods or (b) due to differences in the mutation load carried by the two populations in Germany and in Sri Lanka. If the recovered mutations are considered as 4 (i.e., including the 2 'mutants' that were lost before testing) then we get a figure of 0.12 mutation/mosquito which is closer to the value obtained by VandeHey. Of course, we cannot be absolutely certain that the 2 "mutant" types that were lost before tests on them could be carried out are true mutants.

Compared to *C. pipiens* the mutation frequency in *A. aegypti* is high. Studies on the mutation load in some African populations of *A. aegypti* have been carried out.[5] Two populations from the coast of Kenya gave 2.96 add 2.72 mutation/mosquito, carried in the heterozygous condition. Populations from West Africa gave 0.52 (Ghana), 0.72 (Nigeria) and 0.84 (Congo). Each population carried a distinctive array of different kinds of mutants and in these tests was found to vary widely in the mutation load carried by them.

The amount of hidden genetic variability in a laboratory population of *A. aegypti* (L) has also been assessed.[12] A frequency of 1.32 mutations/ individuals was obtained, which means that in a laboratory colony too, similar loads can be maintained.

The data so far obtained indicate that *A. aegypti* is apparently more variable in morphological characteristics than *C. pipiens* as even the low values for *Aedes* from Ghana, Nigeria and Congo are well above those for *Culex*. This higher incidence of mutations have been attributed to the mating ability shown by *A. aegypti*. VandeHey[12] has referred to the work of Hickey in 1961, which shows that in *A. aegypti* there are numerous morphological differences in populations, with no indication of sterility barriers and that complete crossability exist between populations throughout its distribution.

But recently the work of Tabachnick and Powell[11] have shown contradictory results on the crossing ability of *A. aegypti*. The population genetic analysis of the domestic population of *A. aegypti* in villages near Rabai area near Mombasa, Kenya, has shown that the mosquito population in each village is a panmictic unit with no significant genetic substructuring; and that significant genetic differences exist between villages less than 2 km apart and that gene frequencies are temporaly stable. If the work of Tabachnick and Powell show that interbreeding does not take place between villages 2 km apart, then it would be difficult to explain higher mutation load in this mosquito as been due to out-breeding, as this does not seem to occur.

It may be that the actual observation of a greater variability in the morphological characteristics of *A. aegypti* could be attributed on the other hand to the difference in egg laying habit of *A. aegypti* when compared to *C. pipiens*. In *C. pipiens*, a large number of eggs (200-250) are laid in the form of a single raft by each female, resulting in subpopulations within which there is hardly any difference in the genotype as all the individuals come from the same mother and perhaps the same father. The chance of them mating among themselves may be great. This would lead to homozygosity of the recessive mutants on which selection can operate. In contrast with this egg laying habit, *A. aegypti* females lay a few eggs separately one at a time. No group formation can occur and the possibility of mating between individuals of different genotypes would be considerably greater than for *Culex*. This would obviously lead to a greater heterozygosity at genetic sites in *A. aegypti* than in *Culex*.

The heterozygote mutant load has been worked out for other Diptera, as well. Craig and Hickey[5] have tabulated the mutation loads in populations of certain Diptera estimated by a number of authors, as measured by frequency of recovery of visible mutations through inbreeding, and it becomes clear that great variability in the frequency of mutation/individual is present between species even within the same genus. The estimate of 2.38 for a population of *Drosophila hydei* is the highest value for *Drosophila*. This value is well below the average of 2.48 reported for the Kenya populations of *A. aegypti*. In the *C. pipiens* the value of 0.16 though well below those demonstrated for *A. aegypti* and the lowest recorded for any species of mosquitoes, yet demonstrates the occurrence of genetic variability in the natural population.

The data available in the housefly show that the load of hidden heterozygosity is high and differ between the populations.[10]

The mutation frequency/mosquito we have obtained for Sri Lanka population of *C. fatigans* seems to be the lowest of them all, (0.06 mutation/mosquito). But if the two mutants we lost are also considered as true mutants, then our value (0.12 mutation/mosquito) becomes comparable to that obtained by VandeHey for Germany.

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