Research Article

Genetic and Linkage Studies of New Autosomal Sex-Limited Mutant Hairless Antenna in *Anopheles stephensi* Liston

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Received 29 August 2015; Revised 19 November 2015; Accepted 23 November 2015

Academic Editor: Fedai Erler

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A new morphological mutant hairless antenna (*hla*) was induced in the mosquito *Anopheles stephensi* by γ -irradiation. The expression of the mutant in the male adult revealed the antennae without the hairs. The inheritance pattern from our data demonstrates that hairless antenna is essentially sex-limited to males and is autosomal and recessive. The linkage relationships studies between the hairless antenna and greyish brown larva (*grb*) mutant indicated independent assortment and are present on different chromosomes, while the linkage relationship studies between the hairless antenna and ruby eye (*ru*) mutant revealed the suppression of *hla* by the *ru* gene in heterozygous condition. These mutants can be useful in conducting basic and applied research such as construction of linkage maps and understanding biochemical pathways and in genetic control programmes.

1. Introduction

Malaria continues to be a serious threat in many parts of the world including India. Conventional control method, that is, the chemical control, causes two major problems: environmental pollution and insecticide resistance. The insecticides will become less effective due to the resistance. Genetic control is an alternative method of controlling the vectors which includes techniques like sterile insect technique (SIT) and so forth and genetic characterization especially of these species and strains will continue to be an essential component of genetic control strategies aimed at disrupting the transmission of diseases [1].

Anopheles stephensi (order Diptera; family Culicidae) is an important urban malaria vector in the Indian subcontinent and accounts for about 15% of the annual malaria incidence [2]. *An. stephensi* has 3 pairs of chromosomes ($2n = 6$). Three pairs of chromosomes (linkage groups) based on their length and position of the centromere are designated as I (subtelocentric sex chromosomes), II (longest autosome pair), and III (shorter autosome pair) [2, 3].

Traditionally, morphological mutants have been used to construct special genetic load strains containing chromosomal translocations or inversions [4, 5]. Morphological

mutants are extremely useful in understanding the biology, behaviour, and biochemical pathways involved in pigmentation and constructing linkage maps to respective chromosomes and in synthesizing new strains for control of the vector [2]. Genetic markers are necessary for expanding the linkage maps [6]. Mutants obtained by irradiation have been useful in conducting basic genetic research and also applied research. Several radiation induced morphological mutants including wing, eye, and antenna mutants have been reported in *Culex pipiens* [7–10].

Relatively few γ -irradiation induced morphological mutants have been discovered in *Anopheles*. Autosomal hairy (*h*) and sex-linked bubble head mutants were obtained in *An. albimanus* [11], and frizzled (*f*) and homochromyl (*hom1*) were isolated from ^{60}Co -irradiated *An. gambiae* [12].

The present paper describes the induction and isolation of a new radiation induced morphological mutant hairless antenna (*hla*), genetic basis of inheritance, and its linkage relationship with the already available autosomal mutants, that is, greyish brown larvae (*grb*) and ruby eye (*ru*) in *An. stephensi*.

FIGURE 1: Male *Anopheles stephensi*, (a) wild type and (b) hairless antennae mutant (arrow indicating hairless antenna).

2. Materials and Methods

2.1. Mosquito Culture. The larvae and adults were reared in an insectary maintained at $25 \pm 1^\circ\text{C}$ and $75 \pm 5\%$ relative humidity according to the procedure of Shetty [13]. The adults were held in an iron cage (8" X 8" X 8") covered with nylon netting. The adults were fed with 10% glucose solution soaked in cotton. The gravid females were placed in a plastic cup (2.5 × 7.5 cm) lined with filter paper and clean water was placed inside the cage for oviposition. The laid eggs were kept up to 72 hours for complete hatching. The hatched larvae were transferred to water in enamel tray and fed with dried yeast powder and dog biscuits as a source of food.

2.2. Mutants

Hairless Antennae (hla). The hairless antenna mutant was isolated from the ^{60}Co -irradiated mosquitoes of the Subhash Nagar, Mysore (South India) strain. Two- to three-day-old 125 males were irradiated with 3700 rad of ^{60}Co gamma radiation (dose rate is 185 rad/min) at the Kidwai Institute of Oncology, Bangalore. These irradiated males were immediately mass mated with virgin females of the same age. The gravid females were separated individually into plastic vials (2.5 × 7.5 cm) and kept for oviposition. The lines showing less than 50% hatchability were retained and were outcrossed with wild males and females. One of the lines in the F_2 generation, that is, T_{10} , gave 130 eggs and 60 larvae with 47% hatchability. Among the 60 larvae, 30 were males and 28 were females. Out of 30 males, five were hairless antennae males. These males were outcrossed with wild type females and the resulting F_3 progeny were inbred to get F_4 generations. The hairless antenna males were observed in alternate generations, that is, F_2 , F_4 , F_6 , F_8 , and so forth, and thus the mutant was maintained in the laboratory.

Contrary to the wild type male, which has bushy antennae, the mutant has hairless antennae. However, in both cases, the length and mouthparts are similar (Figure 1).

Greyish Brown (grb). The mutant greyish brown (grb) was established in *An. stephensi* in our laboratory. The colour is

expressed in the late 1st instar larvae and persists throughout the larval and pupal stages. The mutant colony is vigorous requiring no more care than wild type. The gene "grb" is an autosomal recessive with uniform expression and complete penetrance.

Ruby Eye (ru). The mutant ruby eye was spontaneous in origin. The ruby colour of the eye is expressed in all the developmental stages and adults. The gene "ru" is monofactorial in nature and is autosomal. This is a good marker for *An. stephensi*. The said mutant is maintained in large population cages in our laboratory [14].

2.3. Crosses. Testing the mode of inheritance of hairless antenna involved five crosses. For all crosses, 25 males and 25 females were mass mated. Cross 1 involved the crosses between the males and females of the wild type. Cross 2 involved the crosses between the hairless antenna males and wild type females. Cross 3 involved the F_1 heterozygote mated *inter se*. Cross 4 involved the F_1 heterozygote females being backcrossed to the hairless antenna males. Cross 5 involved the crosses between the F_1 heterozygote females with wild type males.

2.4. Linkage Studies. To study the linkage relationship between hairless antenna and greyish brown larva mutant, pure laboratory strains of the mutants were used. The hairless antenna males having the wild type larva body were crossed with greyish brown larva females. A total of 6 crosses were carried out to study the linkage relationship. The F_1 progeny were inbred to yield F_2 generation and the F_2 progeny were analyzed for wild type, hairless antennae, greyish brown larva, and double mutant (hairless antenna and greyish brown larva) individuals. The same procedure was followed to study the linkage relationship between the hairless antenna and ruby eye mutant.

3. Results and Discussion

We have presented the genetic studies on inheritance of hairless antenna and wild type (Table 1). Cross 1 was carried

TABLE 1: Mode of inheritance of hairless antenna (*hla*) in *An. stephensi*.

Sl. number	Male	Crosses	Female	Total eggs	Total larvae	Hatchability (%)	Male	Female	Total	Hairless antennae males	Grand total	χ^2
1	+/+	x	+/+	751	737	98.1	351	357	708	—	708	—
2	<i>hla/hla</i>	x	+/+	487	407	83.6	178	203	381	—	381	—
3	<i>hla/+</i>	x	<i>hla/+</i>	4258	3805	84.0	1370 (1448.25)	1683	3053	561 (482.75)	3614	15.38*
4	<i>hla/hla</i>	x	<i>hla/+</i>	1176	—	—	—	—	—	—	—	—
5	<i>hla/+</i>	x	+/+	595	539	90.6	217	302	519	—	519	—

* χ^2 nonsignificant ($p < 0.05$).
Number in the parentheses: expected number of progeny for that respective cross.
+/+: Wild type.
hla/hla: Hairless antenna.
hla/+: Heterozygous wild type.

out to establish the pure-bred wild type in the laboratory. Cross 2 involved the cross between the hairless antenna and wild type female. All the males and females were normal in the F_1 progeny. In cross 3, part of the F_1 progeny was inbred to get F_2 progeny. The wild type male and hairless antenna mutant occurred in 3:1 ratio of the male progeny of the F_2 progeny, indicating the recessive nature of the gene *hla*. All the females of the F_2 progeny were of wild type, and no significant values were obtained for cross 3. Part of the F_1 heterozygote female progeny were backcrossed to the hairless antenna (cross 4): no larva hatched out from the eggs of cross 4. The microscopic dissection revealed no embryonic development in the eggs. In cross 5, the F_1 heterozygote male progeny was crossed to wild type females in which no hairless antenna males were observed among the F_2 progeny. The heterogametic male progeny of cross 2 involving the hairless antenna and wild type female were of wild type and, therefore, the gene *hla* is autosomal.

Since the mutation affects only the male individuals, the gene *hla* is sex-limited gene. The progeny of the backcross involving the F_1 heterogametic female and hairless antenna revealed only the nonembryonated eggs. The exact mechanism of the lethality of the embryo is not clearly understood. However, the lethality may be due to immobility of male gamete to fuse with an egg or incompatibility of the egg cytoplasm.

Seawright et al. [15] have reported bald antenna (*ba*) in *An. albimanus*, which was sex-limited, autosomal, and recessive. In bald antenna, the remains of the hairs of antennae were presented in the pupal moult, which is not seen in the pupal moult of hairless antenna of *An. stephensi* in the present study indicating that both mutants are different.

The results of the linkage studies between the hairless antenna and the greyish brown larva are presented in Table 2. Cross 1 establishes the pure breed of greyish brown larva. The F_1 progeny of cross 2 involving the hairless antenna and wild type females were only wild type. The F_1 progeny of cross 3 involving the hairless antenna and greyish brown larva parents were of wild type. The results of the cross 4 involving F_1 progeny mated *inter se* showed a near 9:3:3:1 ratio of wild type, greyish brown larva, hairless antenna, and double mutant (greyish brown larva and hairless antennae) among the total male progeny in the F_2 generation indicating independent assortment. The female progeny of the F_2 generation showed 3:1 ratio of wild type and greyish brown larva. All the F_2 progeny of cross 5 resembled wild type and no significant ($p < 0.05$) value was obtained for cross 4 (Table 2). No progeny were obtained from cross 6. It is pertinent to mention here that the backcross carried out between the F_1 heterozygote females with double mutant male (hairless antenna and greyish brown larva) did not produce any eggs indicating sterility or incompatibility. The genes *grb* and *hla* are both autosomal and the present study revealed the nonlinkage between the two genes. Therefore, these two genes are present on different linkage groups.

The results of the crosses between the hairless antenna and ruby eye are given in Table 3. Cross 1 was for establishing pure breed of ruby eye mutant. The F_1 progeny of cross 2 involving the hairless antenna and wild type females

were wild type. All the F_1 progeny of cross 3 involving the hairless antenna and ruby eye parents were wild type. Cross 4 involving F_1 progeny being mated *inter se* did not reveal the normal dihybrid ratio of 9:3:3:1 of the wild type, hairless antenna, ruby eye, double mutant (hairless antenna and ruby eye) among the F_2 male progeny. The actual numbers of males obtained were 52:20:5:5, respectively, for the wild type, hairless antenna, ruby eye, double mutant (hairless antenna and ruby eye). The expected numbers were 46.125:15.375:15.375:5.125. Though the wild type, hairless antenna and double mutant (hairless antenna and ruby eye) occurrence was in the expected ratio, the ruby eye expected ratio varied with observed number. Similarly, the observed female individual ratio of the wild type and ruby eye (97:9) also deviated from the expected ratio (79.5:26.5). These variations may be due to the suppressive nature of the *hla* gene over the *ru* gene. This may be in the heterozygous condition of *hla* gene and homozygous condition of *ru* gene (*hla/+ ru/ru*) individuals. In both deviations, the ruby eye individuals had 1:2 ratio of homozygous population (*+/+ ru/ru*) and heterozygous population (*hla/+ ru/ru*). In the present study, observed suppression of gene activity in the progeny of F_2 generation correlates with the expected 5:10 (15.375) and 9:18 (26.5) of homozygotes: heterozygotes individuals of male and female ruby eye individuals of F_2 progeny, respectively. The progeny of cross 5 were wild type. No larvae hatched out from the eggs of cross 6. For the first time such gene interaction was observed in *An. stephensi* and no such observations are reported so far in other mosquito species.

Genes were tentatively assigned to the linkage groups based on genetic markers in *An. stephensi*. A preliminary report suggested that white eye locus was on the X chromosome (linkage group I), brown palpi, beaked proboscis, wart, and reduced antennae on linkage group II, and 4th costal spot on linkage group III [16]. Assignment of red eye (*r*) to linkage group I, colourless eye (*c*) to linkage group II, and green larva (*g*) and greenish brown larva (*gb*) to linkage group III was tentatively made [17]. Golden-yellow (*gy*) and black larva (*Bl*) mutants were linked with a map distance of 3.75 ± 0.42 and assigned to linkage group III [18]. Sequence of the genes, short palpi (*sp*), diamond palpi (*dp*), black larva (*Bl*), and dieldrin resistance (*DI*) was shown to be as *sp-dp-Bl-DI* and they were assigned to linkage group III [19]. Spotless wings (*sl*) and 2nd-3rd costal spots fused (*2-3f*) have been mapped onto linkage group II, approximately 79.5 map units apart [6]. Three new eye colour mutants, scarlet (*wsca*), red-spotted (*prs*), and pigmentless (*p*), were found to be sex-linked (linkage group I) [20].

Larval body and eye colour mutants have been studied in our laboratory in *An. stephensi*. The eye colour mutants isolated include white (*w*) [2], ruby (*ru*) [14], and sienna (*si*) [21]. Larval body colour mutants include yellow (*y*) [2], dark (*da*) [21], brown (*b*) [22], green (*g*) [2], greyish brown (*grb*) [23], grey (*gy*) [21], greyish black (*gyb*) [24], and green thorax (*gt*) [25].

Linkage studies from our laboratory showed that ruby (*ru*) was nonlinked with greyish brown (*grb*) [23], dark body colour (*da*) [2], and green thorax (*gt*) [25]. Sienna eye (*si*)

TABLE 2: Linkage studies between the hairless antenna (*hla*) and the greyish brown (*grb*) larva in *An. stephensi*.

Sl. number	Crosses		Female	Total eggs	Total larvae	Hatchability (%)	Wild type	Males			Females			Grand total	χ^2
	Male	x						Wild type	Hairless antenna	Greyish brown	Hairless antenna & greyish brown	Wild type	Greyish brown		
1	<i>grb/grb</i>	x	<i>grb/grb</i>	801	757	94.5	—	—	355	—	—	—	387	742	—
2	<i>hla/hla</i>	x	+/+	691	607	87.8	253	—	—	—	—	291	—	544	—
3	<i>hla/hla</i> +/+	x	+/+	715	651	91.0	293	—	—	—	—	305	—	598	—
4	<i>grb/+</i> +/ <i>hla</i>	x	<i>grb/+</i> +/ <i>hla</i>	1158	1037	89.5	229 (247.5)	94 (82.5)	85 (82.5)	32 (27.5)	293 (289.5)	93 (96.5)	386	826	3.97*
5	<i>grb/+</i> +/ <i>hla</i>	x	+/+ +/+	651	583	89.5	269	—	—	—	—	275	—	544	—

* χ^2 nonsignificant ($p < 0.05$).
Number in the parentheses: expected number of progeny for that respective cross.
grb/grb: Greyish brown.
hla/hla: Hairless antenna.
+/+: Wildtype.
grb/+ +/*hla*: Heterozygous wild type.

TABLE 3: Linkage studies between the hairless antenna (*hla*) and the ruby eye (*ru*) in *An. stephensi*.

Sl. number	Crosses		Total eggs	Total larvae	Hatchability (%)	Males				Females		Grand total	χ^2	
	Male	x				Female	Wild type	Hairless antenna	Ruby eye	Ruby eye & hairless antenna	Total			Wild type
1	<i>ru/ru</i>	x	<i>ru/ru</i>	509	481	96.0	—	—	221	—	243	243	464	—
2	<i>hla/hla</i>	x	+/+	691	607	87.8	253	—	—	—	253	291	544	—
3	<i>hla/hla</i> +/+	x	+/+	487	395	81.1	161	—	—	—	161	185	346	—
4	<i>ru/+ +/hla</i>	x	<i>ru/+ +/hla</i>	295	218	73.9	52 (46.125)	20 (15.375)	5 (15.375)	5 (15.375)	82	97 (79.5)	188	24.5*
5	<i>ru/+ +/hla</i>	x	+/+ +/+	585	517	88.4	269	—	—	—	269	255	494	—

* χ^2 nonsignificant ($p < 0.05$).

Number in the parentheses: expected number of progeny for that respective cross.

ru/ru: Ruby eye.*hla/hla*: Hairless antenna.

+/+: Wild type.

TABLE 4: A tentative linkage map of *Anopheles stephensi* based on morphological genetic markers.

Sex chromosome (linkage group I)	Autosome (linkage group II)	Autosome (linkage group III)	Unassigned
White eye (<i>w</i>) [2, 16]	Ruby eye (<i>ru</i>) [14]	Greyish brown body (<i>grb</i>) [23]	Yellow (<i>y</i>) [2]
Scarlet (<i>wsc</i>), red-spotted (<i>prs</i>), and pigmentless (<i>p</i>) eyes [20]	Brown palpi, beaked proboscis, wart, and reduced antennae [16]	Sienna eye (<i>si</i>) [21]	Hairless antenna (<i>hla</i>)
Red eye (<i>r</i>) [17]	Colourless eye (<i>c</i>) [17]	Dark body (<i>da</i>) [21]/grey (<i>gy</i>) [21]/greenish black (<i>gbl</i>) [26]	
		Green thorax (<i>gt</i>) [25]	
		Brown (<i>b</i>) [22]/green (<i>g</i>) [21]	
		4th costal spot [16]	
		Green larva (<i>g</i>) and greenish brown larva (<i>gb</i>) [17]	
		Spotless wings (<i>sl</i>) and 2nd-3rd costal spots fused (2-3f) [6]	
		Short palpi (<i>sp</i>), diamond palpi (<i>dp</i>), black larva (<i>Bl</i>), and dieldrin resistance (<i>DI</i>) [19]	
		Golden-yellow (<i>gy</i>) and black larva (<i>Bl</i>) [18]	
		Greyish black (<i>gyb</i>) [23]	

was found to be closely linked (3.34 ± 1.88 cM) to *grb* [21]. From the present study, the genes *hla* and *grb* were found to be nonlinked and also suppressive nature of *hla* gene over the *ru* was observed.

Our allelic studies showed that brown and green larvae (*g*) [2], grey (*gy*) and greenish black (*gbl*) and dark (*da*), and grey (*ge*) and greenish black (*gbl*) belong to allelic series [21, 26].

All these studies indicate that white eye is present on the sex chromosome, and green thorax, greyish brown, dark body, grey body, greenish black body, and sienna eye are present on one autosome (linkage group), while ruby eye is present on the other autosome (linkage group). Yellow, green, brown larva and hairless antennae could be on either of the autosomes (Table 4).

4. Conclusion

For the first time, the hairless antenna (*hla*) mutant has been established in *An. stephensi*. The genetic studies of inheritance of *hla* clearly showed that it was autosomal, recessive, and sex-limited. The gene *hla* was expressed in males and all the females were normal. The viability of the mutant is good and can be identified very easily from the wild type without the aid of microscope. Hence, the gene *hla* is also considered to be an excellent marker for *An. stephensi*. Linkage analysis between hairless antenna and greyish brown larvae revealed that the genes *hla* and *grb* are nonlinked and could be present on different autosomes, while the data from the crosses between

hla and *ru* revealed that the mutants segregate in non-Mendelian ratio, indicating that *hla* prevents the expression of *ru* in hemizygous condition.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

This work was supported by financial assistance from Department of Science and Technology (DST), New Delhi, to Professor N. J. Shetty.

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