Rapid method for confirming the identity of red eye and rosy eye colour mutants of *Aedes aegypti* mosquito

The use of phenotypic mutants, particularly eye colour mutants, in the genetic analysis of *Aedes aegypti* mosquitoes is a traditional approach. Often, these eye colour mutants are isolated from different populations and are identified on the basis of only morphological examination of eye colour. The major difficulty in identifying these eye colour mutants is non-availability of similar eye colour reference strains for ready comparison. Moreover, it is not always possible to send live material to the laboratories outside the country where reference collections are available. Even if reference strain mutants are available, further characterization of the mutants involves a series of crossing and back-crossing experiments to determine allelism of mutants. This procedure is time-consuming and labour-intensive. Among the *Aedes* eye colour mutants, the red eye and rosy eye cannot be identified by morphological examination. This paper describes a rapid and easy method to identify these two eye colour mutants.

Wild type (black eye, +) *Ae. aegypti* were from a laboratory colony maintained in Pune since 1994. The stock originated from Mysore district, Karnataka, India.

The red eye (rd) strain originated from the Vector Biology Laboratory, University of Notre Dame, Indiana, USA and the colony was maintained in Pune.

The white eye (wt) strain was established from the white eye-mutant isolated from rd stock.

A rosy eye (ry) female was detected in a laboratory colony. Genetic analysis showed that it was rosy-eye mutant from the locus linked to black tarsus, which is on chromosome III (ref. 1).

The mosquitoes were reared in an insectary maintained at 28 ± 2°C and 70–80% RH and purity of the strains was checked in every generation. Crossing experiments between the wild type and rosy-eye mutants were carried out in 30 cm^3 wooden cages with wire mesh.

Adults were collected soon after eclosion and stored at –70°C until assayed. Individual female mosquito heads were cut-off from the thorax. Batches of five heads were selected from the mutant strains, which had almost the same weight. These were separately homogenized in 50 μl of the sample buffer using sterile pestles (Kontes, USA) in eppendorf tubes. The homogenates were centrifuged at 3500 *g* for 5–7 min and 40 μl of the supernatant transferred to clean microtitre plates. Absorbance was measured in a UV Max, Kinetic Plate Reader (Molecular Device, USA) at 405, 450, 490, 570 and 650 nm wavelengths. Forty μl of buffer alone was used as a blank control.

The protein yield was determined in the heads of mosquitoes of more or less the same size and weight by preparing homogenates in phosphate buffer saline (PBS; 50 mM, pH 7.2) by the method described by Lowry *et al.*[2]. A reference standard protein curve was prepared using bovine serum albumin fraction V.

The protein yield did not show any significant difference in these two mutant strains. When the homogenates of five heads prepared in the sample buffer were subjected to spectrophotometry, there were differences at 490 nm in different mutant strains (Figure 1). The optical density (OD) obtained with the wild-type strain was taken as 100% and the other ODs were analysed using the formula: (OD test sample/OD wild type) × 100. Results showed that the values for ry were between 75 and 85, while those for rd were between 40 and 50. It was notable that these differences were not significant when the heads were triturated in normal saline or PBS. When the number of heads was increased to ten in the same volume of sample buffer, the difference was reduced. When the hybrids of ry and wild type were incorporated in the test, it was not possible to distinguish the hybrids from the wild-type strain. Since ry is a recessive character, the hybrids showed an OD close to that obtained with the wild type.

There are five mutants known in *Ae. aegypti* which have red or reddish eyes, viz. red eye (rd), rusty eye (ru), plum eye (ru^{pm}), creamy eye (cr) and rosy eye (ry). Of these, ry, ru and ru^{pm} are sex-linked on linkage group I, while cr and ry are on linkage group II and III respectively[3]. To distinguish mutants on linkage group I, the traditional approach is to perform crosses and test-crosses with the wild-type strain to determine whether the eye colour is sex-linked or not. If it is sex-linked, it eliminates rd, ru and ru^{pm} from the above list. Linkage studies using two to three point crosses can distinguish whether it is rd, ru/or/ru^{pm}. Further confirmation between ru and ru^{pm} needs more complicated crossing and back-crossing experiments or crossing with the reference strains. Whereas cr lacks pigment in the larval and pupal stages, the creamy-grey colour persists without darkening in the adults. Hence it can be distinguished from the rest of the mutants. On the other hand, in ry the eye is pink to rose-coloured and darkens progressively with age in adults, which is also the case with rd. Hence, the difference between rd and ry creates confusion, particularly if the investigator is not experienced or has not seen some of these mutants earlier.

The spectrophotometric analysis of the heads of these mutants is simple to perform. Even mosquitoes stored at –70°C can be used. This method is rapid and easy to use in identifying rd and ry using

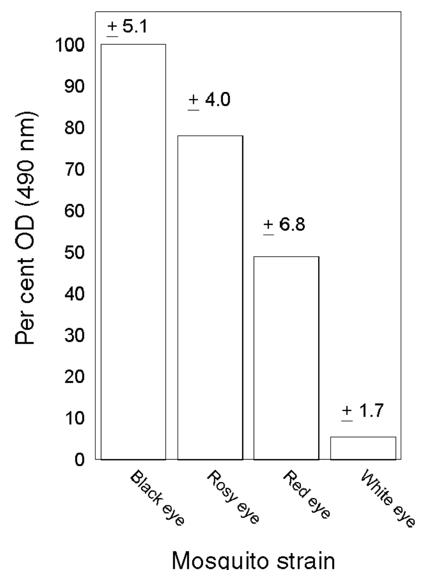

Figure 1. Per cent optical density of homogenates of heads of different strains of *Aedes aegypti*. (Average of 5 replicates and 5 heads in each replicate.)

the wild type as a reference strain. The OD obtained with the heads of white-colour mutant is very close to the OD obtained with the buffer control. This suggests that the colorimetric assay on the homogenates of mosquitoes is primarily due to the eye colour pigments.

The homogenates prepared in PBS do not give a clear distinction, perhaps due to the low solubility of the pigment proteins. The increased amount of proteins while increasing the number to seven heads in 50 μl also creates a problem due to a proportionate increase in the brain protein content. It is not possible to incorporate protein assays in the suggested test due to the interference obtained on account of the ingredients used in the sample buffer. This can easily be overcome by homogenizing the heads of the mutant and wild type of more or less the same size and weight.

We have also used two-dimensional polyacrylamide gel electrophoresis (2D SDS–PAGE) and random amplification of polymorphic deoxyribonucleic acid (RAPD) analysis to distinguish these eye colour mutant strain (results not mentioned here). These two methods also showed different profiles of both the mutants, but they are not reliable since the results would not be reproducible due to likely defences in the different genetic backgrounds of the mutant strains. The rosy eye mutant has also been shown to be low in the susceptibility to Chikungunya virus compared to wild-type strain[1]. Besides this, it has AChE-II allele, which is highly active with the substrate acetylthiocholine iodide as compared to wild-type strain[4]. Therefore based on 2D SDS–PAGE and RAPD it is difficult to distinguish eye colour mutants. Of these three methods, spectrophotometry seems to be simple and rapid which can accurately identify the mutants. The added advantage of using spectrophotometry for this purpose is that the wild-types mosquitoes can be used as reference.

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Effect of global warming on snow ablation pattern in the Himalaya

The Himalaya has a large concentration of glaciers and permanent snowfields. During winter, most of the high-altitude regions experience snowfall, and snow cover plays an important role in the ecology of the region. Melting from seasonal snow cover during summer forms an important source of many rivers originating in the Higher Himalaya. Therefore, understanding of snow accumulation and ablation is important for utilization of the Himalayan water resource.

Snowpack ablation is highly sensitive to climatic variations. Increase in atmospheric temperature can enhance energy exchange between the atmosphere and snowpack. This can increase snow-melting. Investigations suggest that climate of the earth has constantly changed in the course of time, during the past ten million years or so. During this time, the earth has experienced alternate cycles of warm and cold periods. The difference in global mean temperature between the Last Glacial Maximum and the present warm period is about 5°C. However, this slow rate of climate change probably changed in the 20th century due to rapid industrialization. Large emissions of CO_2, other trace gases and aerosols have changed the composition of the atmosphere[1]. This is changing the global radiation budget of the earth–atmosphere system. Investigations carried out by Intergovernmental Panel on Climate Change (IPCC)[2] have concluded that the earth's average temperature has increased by 0.6 ± 0.2°C in the 20th century. This increase in temperature will continue in the 21st century and average surface temperature of the

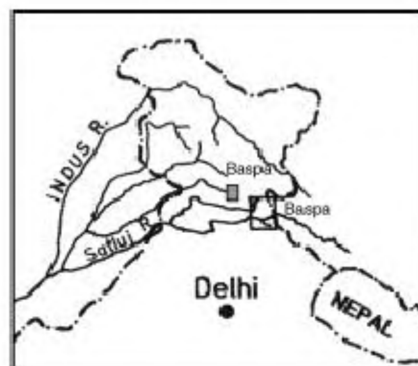

Figure 1. Location map of Beas and Baspa basins, Himachal Pradesh.