

## 1.6 Development of molecular markers for the identification of Biological forms of *Anopheles stephensi* prevalent in arid areas of Rajasthan-

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### OBJECTIVES

1. Identification of biological forms of *Anopheles stephensi* using molecular tools and studies on their bionomics and distribution in different arid environs.

### PROGRESS

The studies during the report period have been carried out in Jodhpur, Jaisalmer and Barmer districts, as per project protocol and the main emphasis has been given on the investigations related to the distribution of different bio-forms of *An. stephensi* present in the study areas. Besides 'type' and variety *mysorensis*, during the studies, an 'intermediate' form was also found having egg ridges 14-15, whereas, the 'type' form and var. *mysorensis* were identified on the basis of egg ridge  $\geq 16$  and  $<14$  ridge count, respectively.

**Table 1. Observations on the morphological details of eggs of different bio-forms of *An. stephensi***

Egg details	Type	Intermediate	Mysorensis
Shape of egg-clutch	Rosette	Linear	Linear
No of ridges	>15	14-15	<14
Ridge type	Overlapped & Notched	Overlapped & Notched	No overlapping & without notch

The arrangement of the eggs on the water surface in case of 'intermediate' form, like var. *mysorensis* was observed in linear fashion, in contrast to 'type' form, where it was found in rosette fashion, however, the ridge type in 'intermediate' form was found to be overlapped and notched, like 'type' form, which in var. *mysorensis* exhibited no overlapping and without any notch (Table 1). It indicates that in 'intermediate' form some of the morphological characters resemble with 'type' form and others to var. *mysorensis*.

The measurements of length and width of about 1602 eggs of three bioforms have been taken to calculate the area of the individual egg and record difference in the length and

width in case of individual bio-form (Table 2). The morphometry of eggs exhibited that the average length and width of the eggs of type form was found more in comparison to both 'intermediate' and *mysorensis* forms and the average area of eggs, calculated of all the three forms also followed the same pattern.

**Table 2. Observations on the length and width of eggs of individual bio-form of *An. stephensi***

Egg Measurements	<i>Mysorensis</i>		Intermediate		Type	
	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation
Length (mm)	0.504	0.020	0.522	0.022	0.535	0.024
Width (mm)	0.171	0.0114	0.176	0.011	0.181	0.013
Area (mm <sup>2</sup> )	0.068	0.006	0.072	0.006	0.076	0.008

The measurement of length of the spiracles of different bio-forms revealed that mean length of the spiracles of 'intermediate' form ranged between the mean lengths of 'type' form and var. *mysorensis* (Table 3). The values of spiracular index of 'intermediate' form, was found lower in comparison to 'type' form, but higher than var. *mysorensis*, which indicates that the 'intermediate' form can withstand more extreme conditions than type form, but lesser in comparison to var. *mysorensis*.

**Table 3. Spiracular index of *An. stephensi* bioforms**

Bio-forms	Length of spiracle (mm) Mean + SD	Spiracular Index Mean + SD
Type	0.119 ± 0.008	8.250 ± 0.627
Intermediate	0.0904 ± 0.004	6.232 ± 0.960
Mysorensis	0.079 ± 0.005	6.037 ± 1.23

During the report period, the 'type' form, in Jaisalmer and Barmer districts, was collected from both urban and rural areas, whereas, in Jodhpur district from urban and peri-urban localities (Table 4). The var. *mysorensis* and 'intermediate' form, both in Jaisalmer and Barmer districts, were collected only from rural areas, however, in Jodhpur district, it was collected from urban and peri-urban localities. The breeding of 'type' form was detected from indoor containers, ground cemented tanks, fountains and ditches, of 'intermediate' form from over-head and ground cattle tanks, and the var. *mysorensis* was found breeding in indoor containers, coolers, ground cemented tanks, clay pots, fountains and cattle tanks (Table 4).

**Table 4. Distribution of different bio-forms of *An. stephensi* in study areas and their breeding habitats**

Districts & breeding habitats	Urban localities/Villages		
	Type	Intermediate	<i>Mysorensis</i>
<b>Jaisalmer</b>	Jaisalmer urban, Tejpala , Raimala, Indra nagar, Hameera & Gomath	Raghwa, Satyaya & That	Satyaya, Chinnu & That
<b>Barmer</b>	Barmer Urban, Kawas, Jasol & Goliya	Baitu, Kalyanpura & Jasol	Kawas, Jasol & Baitu.
<b>Jodhpur</b>	Jodhpur urban: Kheme ka kuan, Fidusar, Public park.	Jodhpur urban: Kheme ka kuan, Public park & Bamba. Jhalamand & Mandore	Jodhpur urban: Kheme ka kuan, Public park & Shastri Nagar. Jhalamand & Mandore,
<b>Breeding habitats</b>	Indoor containers, ground cemented tanks, fountains, & ditches.	Over head and ground cattle tanks	Indoor containers, coolers, ground cemented tanks, clay pot, fountains & cattle tanks

The total protein contents of individuals of different bioforms were estimated to determine the difference among them. For estimation of protein content 10 mosquitoes of each bioforms of same age (one day old) were assayed with total protein assay kit (Bio-Rad). Among the females, the protein content was recorded higher in 'type' form ( $4.689 \pm 0.344$  od/min/mg protein) and lowest in 'intermediate' form ( $4.400 \pm 0.199$  od/min/mg protein), whereas, among the males highest in *mysorensis* ( $4.546 \pm 0.424$  od/min/mg protein) and least in males of 'type' form ( $4.189 \pm 0.216$  od/min/mg protein) (Table 5).

**Table 5. Estimated total protein contents of different bio-forms of *An. stephensi***

Bio-forms	Sex	Estimated Protein Contents (OD/min/microgram protein)	
		Average $\pm$ SD	Range
<b>Type</b>	Male	4.189 $\pm$ 0.216	4.561-3.804
	Female	4.689 $\pm$ 0.344	5.287-4.167
<b>Intermediate</b>	Male	4.321 $\pm$ 0.234	4.817-3.923
	Female	4.400 $\pm$ 0.199	4.742- 4.167
<i>Mysorensis</i>	Male	4.546 $\pm$ 0.424	5.049- 3.991
	Female	4.519 $\pm$ 0.267	5.017- 4.154

The studies on the molecular assays for the identification of different bio-forms are in progress.