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Research paper

Population genetic structure of urban malaria vector *Anopheles stephensi* in India

Richa Sharma [a,1], Arvind Sharma [a,1], Ashwani Kumar [a], Madhulika Dube [b], S.K. Gakhar [a,*]

[a] Centre for Biotechnology, M.D. University, Rohtak, Haryana-124001, India
[b] Department of Statistics, M.D. University, Rohtak, Haryana-124001, India

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ABSTRACT

Malaria is a major public health problem in India because climatic condition and geography of India provide an ideal environment for development of malaria vector. Anopheles stephensi is a major urban malaria vector in India and its control has been hampered by insecticide resistance. In present study population genetic structure of *A. stephensi* is analyzed at macro geographic level using 13 microsatellite markers. Significantly high genetic differentiation was found in all studied populations with differentiation values (F_{ST}) ranging from 0.0398 to 0.1808. The geographic distance was found to be playing a major role in genetic differentiation between different populations. Overall three genetic pools were observed and population of central India was found to be coexisting in two genetic pools. High effective population size (N_e) was found in all the studied populations.

1. Introduction

In spite of the recent scale-up of control programs, malaria still remains a major public health problem in tropical and subtropical countries, including India and its dynamics vary from place to place. Diverse climatic conditions and extensive geography of India provide an ideal environment for malaria vector and parasite (Dash et al., 2012). Malaria is estimated to be prevalent in almost all parts of the country and more than two-third of Indian population lives in malaria endemic zones (Sharma, 1999). Among all anopheline vectors, *Anopheles stephensi* (Diptera: Culicidae) is a principal malaria vector in urban areas contributing 12% to the total malaria cases in India (Adak et al., 2005).

Insecticide resistance of the vector and drug resistance in parasites has also posed a serious threat to current malaria control methods and new innovative strategies are being developed. Resistance to DDT and HCN-dieldrin in *A. stephensi* has been already reported and spread of the insecticide resistance is posing a big obstacle in malaria control in India (Singh et al., 2009).

New malaria control strategies focusing on the vector control needs to be developed as it is being widely acknowledged as important milestone in eradication of malaria (Enayati and Hemingway, 2010; Greenwood, 2009; Takken and Knols, 2009). One of the novel strategies in vector control is genetic manipulation of vector population and rendering them refractory to the parasite (Collins and Besansky, 1994). The recent shift in malaria control strategies has highlighted major gaps in knowledge that are needed to be addressed before application of further strategies. Complete and prior information about the population structure of targeted mosquito species along with knowledge of the forces playing a role in maintaining this genetic structure is crucial to evaluate the viability of various malaria control strategies. It is now well established that basic knowledge of vector ecology, biology and genetics is essential for the development of new innovative methods for effective control of vector population (Ndo et al., 2010).

Despite its importance, very little information is available about population genetic structure of *A. stephensi* in India. Previous studies analyzed population of *A. stephensi* at micro geographic level using microsatellite markers (Sharma et al., 2015; Vipin et al., 2010a,b) and mitochondrial COII gene (Sharma et al., 2014).

The aim of the present study is to analyze population structure of urban malaria vector *A. stephensi* in India at macro geographic level using microsatellite markers. The population genetic data of this vector species could enable us to draw effective vector control strategies in India. Microsatellite markers are used in this study because they are highly variable, densely distributed and have high mutation rate.

2. Materials and methods

2.1. Sample collection, identification and DNA isolation

The adult female *A. stephensi* were collected from six different regions in India (Fig. 1). The collection localities varies from 8.4875° N

* Corresponding author.
E-mail address: surengak@gmail.com (S.K. Gakhar).
[1] Both authors contributed equally.

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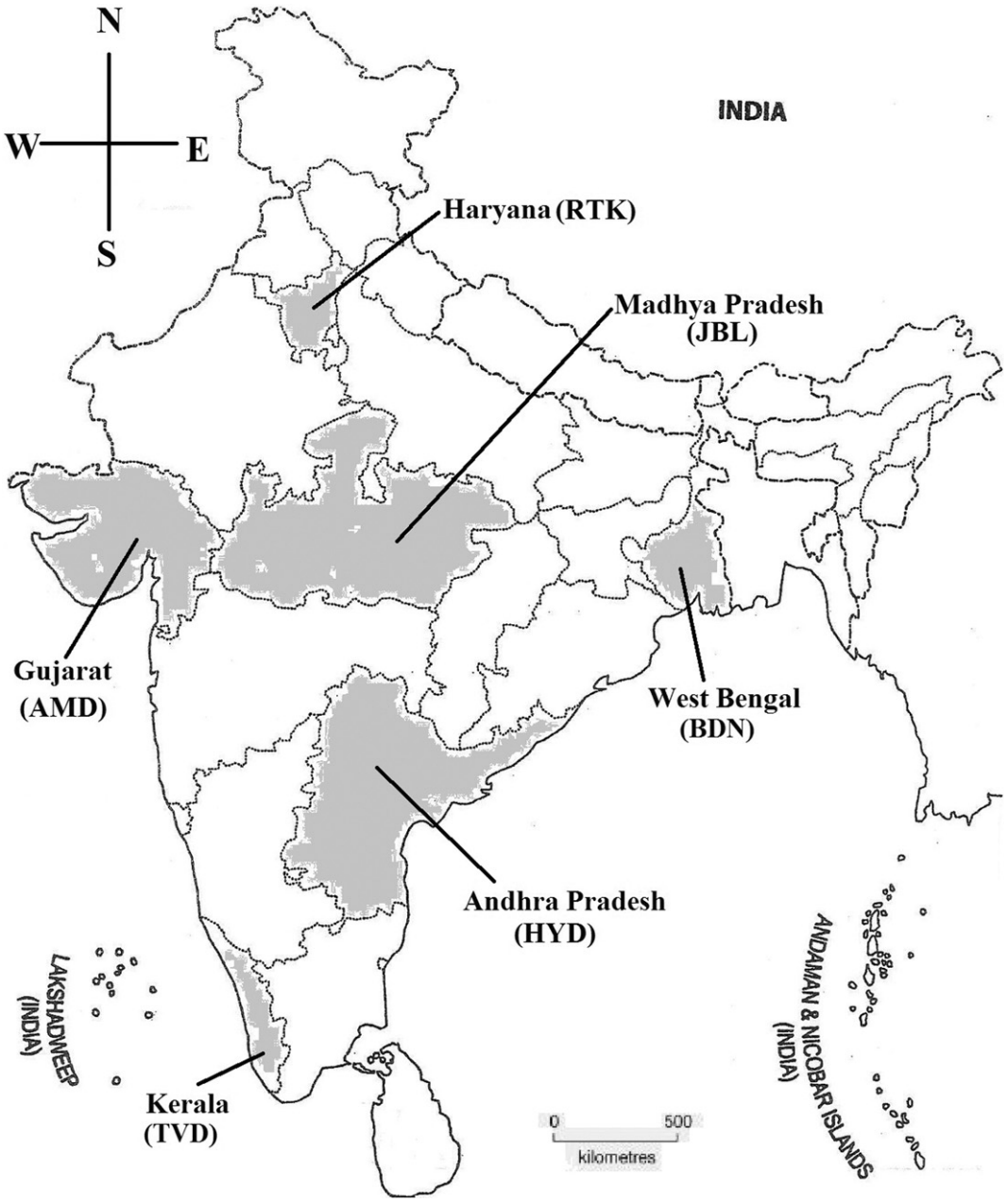

Fig. 1. Map showing collection site of *A. stephensi* mosquitoes from different regions in India.

to 28.8909° N and 72.5800° E to 87.8667° E (Table 1). The geographic distance between different population pairs is given in Table 2. Mosquitoes were collected using a hand held suction tube and torchlight from indoor resting sites mainly human dwelling and cattle sheds. Intact mosquitoes were morphologically identified (Nagpal et al., 2003) and were preserved in 95% ethanol and stored at −20 °C. Genomic DNA of 30 mosquitoes was extracted according to previous protocols (Sharma et al., 2015, Vipin et al., 2010a,b) from the legs and wings of individual mosquitoes and stored at −20 °C.

Table 1
Geographical location of collection sites of *Anopheles stephensi* in India.

Collection site	Code	State	Latitude, longitude coordinates	Sample size	Date
Rohtak	RTK	Haryana	28.8909° N, 76.5796° E	30	August, 2011
Jabalpur	JBL	Madhya Pradesh	23.1672° N, 79.9319° E	30	September, 2011
Hyderabad	HYD	Andhra Pradesh	17.3878° N, 78.4886° E	30	April, 2012
Trivandrum	TVD	Kerala	8.4875° N, 76.9525° E	30	August, 2011
Ahmadabad	AMD	Gujarat	23.0300° N, 72.5800° E	30	March, 2012
Burdwan	BDN	West Bengal	23.2333° N, 87.8667° E	30	August, 2012