Meta-Analysis of Population Genetics of *Aedes vittatus* in India Based on COI Gene: A First Report from a Public Health Point of View

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ABSTRACT
Mosquito-borne diseases profoundly affect public health through the induction of illness, economic burden, and the emergence of epidemics. Efficient control techniques, encompassing vector management, vaccinations, public awareness, and enhanced healthcare infrastructure, are essential for mitigating their impacts. *Aedes vittatus* is increasingly recognised as a possible vector for several viral illnesses, presenting an escalating threat to both humans and animals. Genetic analysis of *Aedes vittatus* is crucial for formulating targeted and sustainable mosquito control tactics. Population genetics research elucidates resistance mechanisms and vector competence, thereby supporting public health initiatives in disease prevention. This study will investigate the genetic diversity and provenance of *Aedes. vittatus* populations gathered from several regions in India to address this gap. The collected data will be essential for enhancing comprehension and management of this species. This research examined the genetic diversity of *Aedes vittatus* populations utilising the DNASp software tool. Haplotype diversity (Hd), nucleotide diversity (π), the average number of pairwise nucleotide changes, and the counts of synonymous and non-synonymous mutations were analysed. Neutrality tests, such as Tajima's D, Fu and Li's D+ and F+, and R2 statistics, were performed. Fifteen sequences were obtained from GenBank, revealing seven haplotypes (H = 7) and a haplotype diversity of 0.819. The sequencing investigation indicated that of the 933 nucleotides analysed, 59.31 were synonymous and 240.69 were non-synonymous. The mean pairwise nucleotide differences (k) was 11.124, although the nucleotide diversity (π) was very modest at 0.03708. The research found 43 polymorphic sites (S = 43) and documented a total of 43 mutations (Eta = 43). Analysis of pairwise nucleotide differences revealed 43 segregating sites. Harpending's raggedness measure (R_2 = 0.1126) lacked statistical significance ($P > 0.05$), suggesting demographic stability among *Aedes vittatus* populations in India. Fu and Li's D+ test value (1.41960) was statistically significant ($P < 0.05$), however Fu and Li's F+ test value (0.87642) was not statistically significant ($P > 0.10$). Furthermore, Fu's F statistic (3.499) was positive, indicating the influence of balanced selection in preserving genetic diversity. Strobeck's S statistic was 0.093, although Tajima's D value (-0.68003) lacked statistical significance ($P > 0.10$). The predicted shape parameter for the discrete Gamma distribution was 1.4123. The Tamura-Nei model (+G) was employed to simulate evolutionary rate variations among sites, incorporating five substitution rate categories with mean evolutionary rates of 0.18, 0.46, 0.78, 1.24, and 2.35 substitutions per site. The nucleotide composition of Ae. vittatus COI sequences was: A = 29.83%, T/U = 39.43%, C = 15.55%, G = 15.18%. Genomic research underscores the impact of evolutionary forces on genetic diversity, with balanced selection maintaining stability in *Aedes vittatus* populations. Some genetic areas change slowly due to functional restrictions, whereas others acquire mutations rapidly, indicating dynamic flexibility. Comprehending these genetic patterns is crucial for evaluating the evolutionary potential of *Aedes vittatus*, especially regarding its adaptation to environmental changes and its involvement in disease transmission. These insights are essential for public health, underscoring the necessity for ongoing genetic research to guide vector control measures and avert mosquito-borne illness outbreaks.

Introduction

Mosquito-borne diseases have a considerable impact on public health by generating illness, economic pressure, and epidemics. Effective control methods, such as vector control, immunisation, public awareness, and improved healthcare systems, are required to reduce their impact. *Aedes* (Fredwardsius) mosquitoes are one of the most deadly mosquito species due to their potential to spread many viral infections, adaptability to human surroundings, and aggressive biting behaviour. Controlling *Aedes* populations through vector management, public awareness, and vaccine campaigns is crucial for preventing large-scale outbreaks. Among the various *Aedes* mosquito species, *Ae. vittatus* (Bigot, 1861) is receiving attention as an emerging vector of multiple viral illnesses [1] and can be a major nuisance to both humans and animals. Because of its aggressive attitude and striking markings, *Ae. vittatus* is an essential species to monitor for public health and pest control purposes. *Ae. vittatus* was initially discovered in Corsica, a Mediterranean island located southeast of France and west of Italy. Over time, its existence has been documented in all five African regions, including Senegal, Sudan, Ethiopia, and Kenya, as well as in Asia, including China, Bangladesh, Iran, Nepal, India, Vietnam, Malaysia, Saudi Arabia, Sri Lanka, and Thailand. Its distribution in Europe is still limited to the western Mediterranean, with records from France, Italy, Portugal, and Spain. This mosquito species is recognised by a dark beak covered in pale yellowish scales. Its scutum has three pairs of small white dots, and its scutellum is covered in white scales over all three lobes, which distinguishes it from other Aedine mosquitos. *Ae. vittatus* has a big, median white spot on tergum I, and its legs are distinguished by dark tibias with a subbasal white mark [2]. The appearance of three pairs of small, spherical, silvery-white spots on the scutum is the most distinguishing feature of this species, distinguishing it apart from other members of the *Aedes* genus. *Ae. vittatus* is noted for its aggressive biting activity and is frequently found in close contact to people and animals. This aggressive biting behaviour contributes to its potential as a vector for a variety of diseases. It prefers human blood above other possible hosts like cattle, pigs, and chickens [3]. *Ae. vittatus*, in addition to the well-known *Ae. aegypti* and *Ae. albopictus*, is found throughout India and can spawn in a range of environments, including tree holes, cement tanks, rock pools, abandoned containers near human settlements, and marsh pools [4]. This invasive plant has successfully spread across several continents, including Africa, Asia, Latin America, and Europe [5]. *Ae. vittatus* is extremely anthropophilic, with a significant affinity for human hosts [6]. It thrives in peridomestic habitats near human settlements, as well as sylvatic conditions within forested areas. Furthermore, this species is an important vector for the transmission and maintenance of several medically significant arboviruses, including yellow fever virus (YFV), dengue virus (DENV), and chikungunya virus (CHIKV) [1].

This demonstrates *Ae. vittatus'* ecological adaptability, since it can thrive in both human-influenced and natural environments. Its position as a vector for dangerous diseases emphasises the public health importance of understanding its behaviour and dispersion. Understanding *Ae. vittatus'* behaviour and distribution is critical for designing effective disease control techniques. This understanding can help to reduce the dangers associated with arbovirus transmission in both urban and rural areas. The presence of these viruses in wild-caught specimens provides compelling evidence of their involvement in maintaining virus circulation in nature [7, 8]. This species is causing increasing fear due to its capacity to adapt to many habitats, including urban areas, which raises the potential of disease spread. Its widespread distribution raises worries about potential epidemics in locations where these viruses are uncommon.

Genetic analysis of *Ae. vittatus* is required for successful mosquito control and illness prevention. Understanding population genetics, resistance mechanisms, and vector competency enables public health organisations to develop more targeted, long-term control measures. Emerging technologies such as gene editing and genetic manipulation provide prospective long-term mosquito management strategies. These innovative technologies may lower mosquito populations or alter their ability to spread diseases, improving public health outcomes. Although mosquito genetic variety, notably *Ae. aegypti*, has been widely investigated globally [9, 10], research on the distribution and genetic variation of *Ae. vittatus* in India is limited [11, 12]. To close this gap, the current study will look into the genetic diversity and origins of *Ae. vittatus* populations collected from several regions in India. The information gathered will be critical to better understanding and managing this species. Furthermore, the findings will help to improve identification procedures and establish more effective control tactics for *Ae. vittatus* in India. This research is critical because it will improve our understanding of the ecological dynamics of *Ae. vittatus*, leading to better pest management strategies.

Material and Method

Data Analysis

Haplotypes were examined utilising the DNAsp software package [13], which computed haplotype diversity (Hd), nucleotide diversity (π), the mean number of pairwise nucleotide differences, and the counts of synonymous and nonsynonymous mutations.

Relationships between haplotypes were examined by calculating parameters according to accepted approaches [14].

Population expansion

To examine neutral mutation, we determined Tajima's D, Fu and Li's D+ and F+, and R_2 statistics using DNAsp software [13]. Tajima's D was calculated based on the number of different sites. Fu's Fs statistic was used to assess the demographic stability [15].

Results

Table 1 Fifteen number of sequences with accession number from India available in GeneBank

Serial Number	Location	Accession Number
1	Odisha, Kalahandi	PQ477920.1
2	Odisha, Ganjam	OR879749.1
3	Kolkata	PQ483326.1
4	Kolkata02	PQ483327.1
5	Kolkata03	PQ483325.1
6	Kolkata04	PQ483324.1
7	Tamilnadu	OP317577.1
8	Tamilnadu02	OL851671.1
9	Tamilnadu03	MZ828135.1
10	Tamilnadu04	MK243685.1
11	Coimbatore, Tamilnadu05	KR872404.1
12	Kerala01	MW931755.1
13	Kerala02	MT858330.1
14	Kerala03	MK491498.1
15	Pondicherry	AY834246.1

Table 2 Genetic variability indices

Number of sequences	Number of sites	Number of segregating sites (polymorphic)	π	K_a	K_b	Θ_s	Θ_g	Number of haplotypes (H)	Haplotype diversity (Hd)	Variance of haplotype diversity (Vhd)
15	933	43	0.03708	11.124	11.124	0.04634	13.224	7	0.819	0.00668

π Nucleotide diversity, K_a Average number of pairwise nucleotide differences, K_b Average number of nucleotide differences, Θ_s Theta (per site) from eta, Θ_g theta (per sequence) from eta

Table 3Population expansion indices

D^+	F^+	F_s	S	D	R_2
1.419602	0.876423	3.499127	0.093	-0.680031	0.112562

D^+ Fu and Li's D test statistic, F^+ Fu and Li's F test statistic, F_s Fu's F_s statistic, S Strobeck's S statistic, D Tajima's D, R_2 Harpending's raggedness statistic

A total of 15 sequences were retrieved from GenBank as shown in **Table 1**, and the sequencing study identified seven haplotypes (H= 7), with a haplotype (gene) diversity (Hd) of 0.819, a haplotype diversity variance of 0.00668, and a standard deviation of haplotype diversity of 0.082.Among the 933 nucleotides of *Ae. vittatus* examined, 59.31 were synonymous sites, while 240.69 were non-synonymous sites. The average number of pairwise nucleotide differences (*k*) was 11.124. The nucleotide diversity (π) of *Ae. vittatus* sequences, based on the COI gene, was found to be low at 0.03708. The number of polymorphic (segregating) sites was 43 (*S* = 43), with a total number of mutations (Eta) also recorded as 43 (**Table 2**).
The results from the pairwise nucleotide differences test indicated 43 polymorphic (segregating) sites. Harpending's raggedness statistic (*R2* = 0.1126) was not significant (*P*> 0.05) across *Ae. vittatus* populations in India. The average values for Fu and Li's *D*+ (1.41960) were significant (*p*< 0.05), while Fu and Li's *F*+ (0.87642) test values were not significant (*P*> 0.10) (**Table 3**). Additionally, Fu's *F* statistic (3.499) was positive. Strobeck's *S* statistic value was 0.093, and Tajima's *D* value was -0.68003, which was not statistically significant (*P*> 0.10) (**Table 3**).The estimated value of the shape parameter for the discrete Gamma distribution was 1.4123 for Indian *Ae. vittatus*. The substitution pattern and rates were estimated under the Tamura-Nei model (+G), using a discrete Gamma distribution to model evolutionary rate differences among sites (five categories, [+G]). The mean evolutionary rates in these categories were 0.18, 0.46, 0.78, 1.24, and 2.35 substitutions per site.The nucleotide frequencies were recorded as: A = 29.83%, T/U = 39.43%, C = 15.55% and G = 15.18%.

Discussion

The recent phylogenetic analysis by Panigrahi et al. [16] indicated that Indian strains of *Ae. vittatus* exhibit similarities to the Pakistan strain of *Ae. cogilli*. Fifteen COI genes of *Ae. vittatus*were obtained from GenBank, resulting in the identification of seven distinct haplotypes. There exists significant genetic variation within the Indian population. This genetic variation indicates a potentially diverse evolutionary history among Indian strains, which may affect their adaptability and response to environmental changes. Comprehending this variation is essential for subsequent research on population dynamics and conservation initiatives. Haplotype diversity (Hd) evaluates the likelihood that two randomly selected sequences from a sample originate from distinct haplotypes. A value of 0.819 indicates significant genetic diversity, reflecting a well-differentiated population characterised by multiple genetic variants. The variance and standard deviation of haplotype diversity quantify the uncertainty associated with the haplotype diversity estimate. The standard deviation of 0.082 indicates a minor variation related to the estimated haplotype diversity. While extensive studies exist on the population genetics of various mosquito species globally [17, 18, 19, 20], there is a lack of information regarding *Ae. vittatus*. The insufficient data on *Ae. vittatus* underscores a deficiency in the comprehension of its population genetics relative to other mosquito species. Additional research may yield significant insights into its genetic diversity and evolutionary dynamics.

The Indian *Ae. vittatus* comprised 933 nucleotides. The mean value of pairwise nucleotide differences (k = 11.124) indicates the average count of nucleotide variations. A value of 11.124 suggests a moderate degree of genetic differentiation among the sequences, highlighting evolutionary changes along with potential population diversity. The analysis of the COI gene in *Ae. vittatus* reveals a low level of nucleotide diversity (π = 0.03708). This low value indicates minimal genetic variation among the sequences at the nucleotide level. *Ae. vittatus* indicates a possible bottleneck effect, recent population expansion, or a comparatively low mutation rate in this mitochondrial marker. The factors identified could lead to a decrease in genetic variability, potentially affecting the species' ability to adapt and respond to environmental changes. Understanding these dynamics is essential for conservation initiatives and forecasting the species' responses to impending ecological challenges. The quantity of polymorphic (segregating) sites within the COI region is constrained. This indicates that there are 43 variable sites within the gene across the sequences. These locations indicate instances where a mutation has been observed within the population. The limited quantity of polymorphic sites shows a minimal degree of genetic diversity present within the population. Investigations into *Ae. aegypti* and *Ae. albopictus* have revealed a moderate to high level of nucleotide diversity in COI, particularly in areas where their range has expanded as a result of human activities [21]. The findings highlight the critical need for monitoring genetic diversity to understand the potential resilience of species in the face of environmental changes. The influence of malaria control actions in India on the genetic diversity of *Ae. vittatus*. Various vector control strategies may cause selective pressures on mosquito populations, resulting in genetic variations. This highlights how particular interventions can inadvertently

affect the genetic composition of mosquito populations, potentially influencing their ability to adapt to changing environments and likely hampering the species' capacity to respond to environmental changes, or it may enhance their suitability for a dynamic environment. In the future, it may represent a considerable oversight by humanity.Understanding these dynamics is essential for formulating effective and sustainable strategies for malaria control. The observed moderate genetic variability in *Ae. vittatus* in India indicates that the species is undergoing adaptation to environmental forces and control strategies, reflecting a balance between homogeneity and diversity. Investigations into mosquito populations reveal that the development of insecticide resistance and changes in environmental conditions play significant roles in the genetic differentiation observed among vector species [22]. Recent investigations from India indicate a moderate level of genetic variability in Indian mosquitoes, which may be attributed to these control programs and the influence of natural selection [23, 24].

Harpending's Raggedness Statistic (R_2: 0.1126, $P > 0.05$) evaluates whether a population has experienced recent expansion or maintains stable. The non-significant finding ($P > 0.05$) indicates that the population has not undergone a recent bottleneck or expansion event. This suggests that the population's size and composition have probably remained stable over time, without substantial variations. Consequently, it suggests a stable demographic history rather than one characterised by fast expansion or decline. Fu and Li's D^+ (1.41960, $P < 0.05$) and F^+ (0.87642, $P > 0.10$) indicate a divergence from neutrality, potentially attributable to purifying selection or demographic alterations, suggesting a lack of strong proof for selective sweeps or population expansion, respectively. Fu's F Statistic (3.499, Positive Value) indicates an abundance of intermediate-frequency alleles, potentially signifying population reduction, balancing selection, or genetic structuring rather than recent expansion. Strobeck's S Statistic (0.093) evaluates genetic variance in relation to population size. A low value (0.093) indicates minimal genetic organisation or differentiation within the Indian *Ae. vittatus* population. Tajima's D (-0.68003, $P > 0.10$, Not Significant): The negative value (-0.68003) indicates an excess of low-frequency polymorphisms, suggesting potential purifying selection or population expansion. The non-significant P-value ($P > 0.10$) indicates that this finding lacks statistical significance. This suggests that although there may be some latent trends in genetic diversity, they lack sufficient strength to yield conclusive insights into the population's evolutionary history. Thus, future research utilising bigger sample sizes or supplementary genetic markers may be required to elucidate these trends. The neutrality and demographic analyses of *Ae. vittatus* COI gene sequences indicate modest genetic variety, a lack of substantial evidence for recent population expansion or bottleneck events, and potential effects of purifying selection or genetic structuring. Research on *Ae. aegypti* indicates increased genetic diversity attributed to its extensive dispersion and adaption to urban settings [25]. In contrast to *Ae. vittatus*, *Ae. aegypti* populations in India frequently exhibit considerable expansion, presumably attributable to heightened urbanisation and climate adaptability [26]. Moreover, selective sweeps resulting from pesticide pressure are more pronounced in *Ae. aegypti*, resulting in diminished genetic variety in specific locations. The effects of urbanisation, social behaviour, and climatic change on *Aedes* mosquitoes in India remain inadequately comprehended. Recent studies underscore difficulties in managing *Ae. aegypti* and *Ae. albopictus* in Western Africa and Thailand owing to little knowledge on their habits [27, 28]. This deficiency in comprehension obstructs effective management tactics, since it constrains the capacity to anticipate how these insects would react to fluctuating surroundings and control approaches. Thus, it is imperative to address the knowledge gaps to formulate targeted interventions that reduce their dissemination and effects.

The Gamma distribution characterises rate variability among nucleotide sites. The nucleotide substitution modelling of *Ae. vittatus* indicates a moderate rate of heterogeneity ($\alpha = 1.4123$), implying that certain sites evolve more rapidly while others are preserved, showing balanced evolutionary changes. Research on *Ae. aegypti* indicates higher heterogeneity in mutation rates, with α values frequently <1 [29], implying a markedly diverse replacement pattern, wherein specific genomic areas develop significantly quicker due to selective forces, notably pesticide resistance. *Anopheles gambiae*, a principal malaria vector, exhibits mutation rate heterogeneity, with α values frequently approaching 1 in numerous genomic areas. Selective pressures, including pyrethroid resistance, have induced rapid localised evolution in genes such as CYP6P3 and VGSC [30]. This suggests that although both mosquito species have variability in their mutation rates, *Ae. aegypti* demonstrates more significant disparities in evolutionary velocity throughout its genome. Conversely, the mutation rates of *An. gambiae* are more consistent, indicating a distinct evolutionary reaction to comparable selection pressures.

The mean evolutionary rates for these five groups are 0.18, 0.46, 0.78, 1.24, and 2.35 substitutions per site. This indicates that various regions of the gene undergo mutations at different rates, with certain sections remaining constant while others experience more frequent changes. The lower values (0.18, 0.46) indicate conserved regions subject to functional constraints. High rates (1.24, 2.35) indicate greater variability in regions, maybe influenced by selecting pressures. This disparity in evolutionary rates suggests that specific gene areas are more essential for functional maintenance, whilst others may exhibit greater adaptability to environmental fluctuations. Thus, the varying rates signify the equilibrium between conservation and adaptation in the gene's evolution. The evolutionary rate for conserved genes in *Culex quinquefasciatus* is low (~0.2–0.5 substitutions per site), close to the lowest category observed in *Ae. aegypti* [31]. This indicates that certain genes in *Cu. quinquefasciatus*retain a consistent function across time, while others may experience alterations that enhance adaptability to environmental conditions. Consequently, these evolutionary dynamics underscore the complex interplay between gene stability and adaptive capacity across many species. Genes associated with pesticide resistance, odorant receptors, and immunological response exhibit elevated rates (~1.0–2.8 substitutions per site), similar with the significant diversity observed in *Ae. aegypti* [31]. This indicates that specific genes are more susceptible to mutations, potentially increasing the adaptability of these species to fluctuating conditions, including exposure to pesticides or pathogens. Thus, understanding these genetic variants can elucidate the evolutionary tactics utilised by these mosquitoes in responding to their environments. *Ae. albopictus*, another significant vector, with a genome that exhibits a comparable spectrum of evolutionary rates. Genes with minimal variation have a substitution rate of 0.15 to 0.5 per site; whereas rapidly evolving genes, such as those associated with detoxification and cuticle formation, demonstrate a substitution rate of 1.2 to 2.6 per site [32]. The disparity in substitution rates across various genes illustrates the adaptive strategies that *Ae. albopictus*utilises to survive in many ecological environments. Such insights can help researchers understand how these mosquitoes may adapt to environmental pressures and contribute to their success as disease vectors. The marginally higher evolutionary rate of *Ae. albopictus* relative to *Ae. aegypti* is probably because to its greater environmental adaptability and more comprehensive pesticide resistance mechanisms. This flexibility allows *Ae. albopictus* to utilise varied habitats and resist control measures, making it a serious public health risk. Understanding these evolutionary features is vital for establishing effective techniques to regulate mosquito populations and limit disease transmission.

The nucleotide composition of the COI gene in *Ae. vittatus* is detailed as follows: Adenine (A) comprises 29.83%, Thymine (T) or Uracil (U) constitutes 39.43%, Cytosine (C) accounts for 15.55%, and Guanine (G) represents 15.18%. This composition reveals a greater ratio of A-T base pairs relative to G-C base pairs, potentially affecting the gene's stability and performance. The A-T richness can affect mutation patterns and stability, as A-T base pairs possess fewer hydrogen bonds than G-C pairings, allowing them more susceptible to mutations. This increased sensitivity to mutations can result in enhanced genetic diversity, potentially influencing the evolutionary processes of mitochondrial genes.The COI gene composition of *Ae. vittatus* closely resembles that of other mosquito species. As is customary with mitochondrial genes, the A-T content is consistently higher in all species, accounting for around 68% to 70% of the total composition [33]. The C-G concentration consistently remains low, roughly 30-32%, across all species. The percentages for *Ae. vittatus* closely resemble those of *Ae. aegypti* and *Cu.quinquefasciatus* [34, 35]. This indicates that the mitochondrial COI genes in mosquitoes have remained unchanged over time.

Conclusion:
Genomic studies elucidate the influence of evolutionary forces on genetic variation, with balanced selection preserving stability in populations significant to public health. Certain parts change gradually due to functional limitations, whereas others swiftly accumulate mutations, indicating dynamic adaptability. Additionally Comprehending these patterns is essential for examining the evolution of *Ae. vittatus* and its ability to adjust to environmental changes, particularly for public health.

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