

Population genetic structure and biology of the malaria vector *Anopheles minimus* A in Vietnam



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Background: The important economic and social implications caused by malaria in Southeast Asia have prompted governments to make this disease a public health priority and to implement national malaria control programmes including disease management and prevention. In Vietnam, malaria mortality and morbidity reached alarming proportions in 1991. Political commitment to malaria control and internal and external funding resulted in a considerable reduction of the malaria burden, particularly in the north. However, malaria control programmes in Southeast Asia are faced with several questions of immediate relevance to implement the control programmes and to consolidate and further improve the malaria situation.

Preventive control strategies in Southeast Asia must deal with the complexity of the vector system caused by the number of species potentially involved in malaria transmission. The follow-up and the evaluation of vector control measures are hindered by the misidentification of vectors due to overlapping morphological characters of the female mosquito, i.e. all major vector species are complexes of species and a number of potential vectors occur in the region that can be confused with the major vectors on the basis of adult morphology. Moreover, the vector species composition and related malaria transmission is influenced by the environmental changes and changes in human practices. National Malaria Control programmes in Southeast Asia must deal with this complex situation in a changing environment where evaluation and possible reassessment of control practices is essential. Furthermore, the appearance of insecticide resistance may reverse the effect of years of effort. Consequently, knowledge of vector resistance and changing trends of resistance in target species are basic requirements to guide insecticide use in malaria control programmes. Understanding the population structure and gene flow of the anopheline mosquitoes, providing insights into the potential spread of insecticide resistance, is therefore essential.

An important widespread malaria vector taxon in Southeast Asia is *Anopheles minimus* s.l. On the Southeast Asian mainland two species in the *An. minimus* complex are commonly recognised, informally designated *An. minimus* A and C. In rural areas, *An. minimus* breeds along the banks of small clear-water streams, yet in the suburbs of Hanoi, northern Vietnam, there is an *An. minimus* population whose immature stages develop in water tanks.

Objectives: This study aimed (1) to evaluate the taxonomic status of the *An. minimus* complex in Vietnam and particularly the status of the urban *An. minimus* population from Hanoi, (2) to assess the population structure and the behaviour of the species within the complex and (3) to develop a reliable method to identify this vector taxon.



Design: Rural *An. minimus* s.l. populations were sampled in 1995, in four villages in northern Vietnam and from 1998 till 1999 in one village in northern Vietnam and in south-central Vietnam each. The urban population, from the suburbs of Hanoi (northern Vietnam), was sampled in 1999. Larval collections and different adult collection methods were used. Allozyme electrophoresis was used to clarify the species composition of the *An. minimus* complex and to study the population genetic structure of *An. minimus* A. Genetic differentiation between the collection methods was surveyed. For one rural study site from northern Vietnam temporal variation in allele frequencies was evaluated over the period 1995-1999. Hierarchical *Fst* values were calculated within and among the study sites and Prevosti distances between samples were subjected to three-dimensional non-metric multidimensional scaling. Collection results were subjected to a correspondence analysis in order to assess the inter- and intraspecific behavioural divergences of *An. minimus* A.

Result and discussion: In rural northern Vietnam two sympatric species within the *An. minimus* complex have been found, similar to species A and C known from Thailand. The population from the suburbs of Hanoi was identified as *An. minimus* A. Significant genetic differentiation was observed between rural and urban *An. minimus* A populations. Ecotypic selection is recognised as an important force in differentiating mosquito populations. However, on the basis of these data and on data from ITS2 rDNA sequencing, there is no evidence for a reproductively isolated status of the urban and the rural populations. However, we recognise the need to extend the study using other molecular loci to further evaluate the taxonomic status of the urban population.

In northern Vietnam no genetic differentiation in *An. minimus* A has been observed among four villages up to 50 km apart. Furthermore, no temporal variation in allele frequencies was observed during the period 1995-1999, indicating that no extreme *Ne minima* in the *An. minimus* A population occurred. During this period no vector control measures were implemented in the study village and seasonal fluctuations do not seem to have influenced substantially the demography of *An. minimus*. In both northern and south-central study villages no genetic differentiation has been found between mosquitoes collected by different methods. Limited macrogeographical genetic differentiation was observed between two rural *An. minimus* A populations at distances of more than 1000 km. Consequently, geographical distance is not the primary factor in differentiating *An. minimus* A populations having the typical breeding ecology.

Large intraspecific behavioural differences were observed among populations of *An. minimus* A. These populations belong to the same species on the basis of the applied genetic markers (allozyme electrophoresis or PCR-RFLP) and showed limited genetic differentiation. In northern Vietnam, species A tended to be more zoophilic, whereas in the study sites of south-central Vietnam it showed marked antropophilic behaviour when cattle was scarce. Hence, *An. minimus* A is able to change its host preference in function of local situations in host availability. In the most northern study site, *An. minimus* A showed noteworthy endophilic behaviour. The observed intraspecific behavioural differences in *An. minimus* A are consistent with the earlier observations that the existence of the cryptic species, A and C, did not resolve all observed behavioural heterogeneities within *An. minimus* s.l.

An identification method was developed for *An. minimus* species A and C, and four related species, *An. aconitus*, *An. pampanai*, *An. varuna* and *An. jeyporiensis*. These closely related species, playing different roles in malaria transmission, are difficult to distinguish from the members of the complex on the basis of adult morphology, so vector and non-vector species can easily be confused. PCR-amplified internal transcribed spacer 2 (ITS2) ribonuclear DNA (rDNA) fragments were digested with restriction endonuclease BsiZI. Clear diagnostic banding patterns



for the six species were obtained on agarose gels. Testing field-collected specimens from different regions in Southeast Asia indicated that the technique will be applicable over a wide geographic area.

Morphologically identified *An. minimus* collected in Binh Thuan Province, Vietnam turned out to be *An. varuna* by PCR-RFLP. Morphologically identified *An. minimus* s.l. mosquitoes originating from Rattanakiry Province, Cambodia, showed a restriction pattern that was clearly different from known diagnostic banding patterns. Specimens having this deviating pattern showed to be *An. culicifacies* B, previously unknown to occur in Cambodia. *An. varuna* and *An. culicifacies* B are not considered as malaria vectors, but they were confused with the malaria vector *An. minimus* A based on adult morphology. These two examples clearly show the importance of improved vector species identification in unravelling the vector system in Southeast Asia.

Conclusion: PCR-RFLP turned out to be a reliable method for the identification of *Anopheles* species in Southeast Asia. This assay has the advantage that PCR amplification should always produce a product, and that RFLP profiles different from known diagnostic banding patterns can be detected. This is clearly advantageous in the context of Southeast Asia where adult overlapping morphology of *Anopheles* mosquitoes can obscure our knowledge of vector distribution. The assay showed the ability to detect species previously unknown to occur in the region, and to identify correctly up to seven closely related species. Moreover, the same PCR-RFLP assay proved to be useful in identifying the members of the *An. funestus* group.

Geographical distance is not the primary factor differentiating the *An. minimus* A populations, whereas the type of larval habitat seems to enhance population differentiation. The limited macrogeographical population structure of *An. minimus* A could indicate that genes can spread over large areas. This is especially important in the light of the spread of insecticide resistance. However, the effect of the shallow population structure of the rural populations on contemporary gene flow should be further evaluated. The presence of an 'urban' *An. minimus* A population shows the ability of this species to adapt to anthropogenic environmental changes. Moreover, *An. minimus* A is able to change its host preference in function of local situations in host availability. Hence, its role in malaria transmission can differ from region to region. Similarly, the impact of vector control on this species may differ between localities.

Finally, this study showed that intraspecific behavioural differences in *Anopheles* species can occur and that these behavioural heterogeneities, albeit important for disease transmission and control, are not *a priori* indicative for the presence of cryptic species. Contemporary and future control strategies have to take into account that the impact of vector control can vary among populations of the same species.