


## Article

# Time of Test Periods Influence the Behavioral Responses of *Anopheles minimus* and *Anopheles dirus* (Diptera: Culicidae) to DEET

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† This paper is dedicated to the memory of Dr. Michael J. Bangs who passed away on 9 March 2021.

**Simple Summary:** The influence of environmental and physiological factors on various aspects of normal mosquito behavior is unclear. The efficacy of repellents against mosquitoes depends on the vector species as well as the concentration and formulation of the repellent. In this study, we observed the behavioral responses of two night-biting malaria vectors in Thailand, *Anopheles minimus* and *Anopheles dirus*, during daytime and nighttime. We demonstrated that time of observation has a considerable impact on behavioral responses for both species. When optimizing an excito-repellency assay system, time of observation-based testing should be considered in order to prevent an under- or overestimation of behavioral responses.

**Abstract:** Information on factors influencing the behavioral responses of mosquitoes to repellents is lacking and poorly understood, especially in the *Anopheles* species, night-biting mosquitoes. Our goal was to investigate the impact of different time periods on circadian activity and behavioral responses of two malaria vectors, *Anopheles minimus* and *An. dirus*, to 5% DEET using an excito-repellency test system. Each mosquito species was exposed to the repellent during the daytime (06.00–18.00) and nighttime (18.00–06.00), and time of observation was further divided into four 3-h intervals. Significant escape responses were observed between daytime and nighttime for *An. minimus* in both noncontact and contact tests. *An. dirus* showed statistical differences in contact irritancy escape response, whereas no significant difference was found in noncontact repellency tests. Both mosquito species showed more significantly higher escape responses when exposed to DEET during the afternoon and late in the night. This finding indicates that the time of testing may affect the behavioral responses of mosquitoes to repellents, especially in *An. minimus* and *An. dirus*. A better understanding of nocturnally active mosquito behavioral responses spanning from dusk to dawn would assist in optimizing product development, screening, and effective evaluation.

**Keywords:** *Anopheles minimus*; *Anopheles dirus*; time of test; avoidance behavioral response; DEET



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## 1. Introduction

Malaria is an illness caused by *Plasmodium* parasites. The parasite is transmitted by competent *Anopheles* mosquitoes to humans. It continues to be a serious problem worldwide, including in some provinces in Thailand, especially near international borders with Myanmar and Cambodia, where malaria is endemic [1,2]. These provinces are