Journal of Medical Entomology, 58(6), 2021, 2107–2113
doi: 10.1093/jme/tjab105
Advance Access Publication Date: 9 June 2021
Research

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Forced Egg Laying Method to Establish F1 Progeny from Field Populations and Laboratory Strains of *Anopheles* Mosquitoes (Diptera: Culicidae) in Thailand

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Subject Editor: Nobuko Tuno

Received 23 January 2021; Editorial decision 17 May 2021

Abstract

Successful monitoring of physiological resistance of malaria vectors requires about 150 female mosquitoes for a single set of tests. In some situations, the sampling effort is insufficient due to the low number of field-caught mosquitoes. To address this challenge, we demonstrate the feasibility of using the forced oviposition method for producing F_1 from field-caught *Anopheles* mosquitoes. A total of 430 and 598 gravid *Anopheles* females from four laboratory strains and five field populations, respectively, were tested. After blood feeding, gravid mosquitoes were individually introduced into transparent plastic vials, containing moistened cotton balls topped with a 4 cm^2 piece of filter paper. The number of eggs, hatching larvae, pupation, and adult emergence were recorded daily. The mean number of eggs per female mosquito ranged from 39.3 for *Anopheles cracens* to 93.6 for *Anopheles dirus* in the laboratory strains, and from 36.3 for *Anopheles harrisoni* to 147.6 for *Anopheles barbirostris* s.l. in the field populations. A relatively high egg hatching rate was found in *An. dirus* (95.85%), *Anopheles minimus* (78.22%), and *An. cracens* (75.59%). Similarly, a relatively high pupation rate was found for almost all test species ranging from 66% for *An. minimus* to 98.7% for *Anopheles maculatus*, and lowest for *An. harrisoni* (43.9%). Highly successful adult emergence rate was observed among 85–100% of pupae that emerged in all tested mosquito populations. The in-tube forced oviposition method is a promising method for the production of sufficient F_1 progeny for molecular identification, vector competence, insecticide resistance, and bioassay studies.

Key words: force, egg laying, oviposition, F1 progeny, *Anopheles* mosquito

Malaria is the main cause of morbidity and mortality in Thailand with around 5,000 annual cases in 2019 treated in the public health system alone (BVBD 2019). Malaria control in Thailand relies mainly on vector control through the use of insecticide-treated nets, long-lasting insecticide nets (LLINs), and indoor residual spraying mostly in regions of perennial and seasonal transmission. The success of such interventions requires a good knowledge of vector populations particularly their susceptibility status to the main insecticides used for such control program in order to detect and monitor resistance to these insecticides. This requires the ability to test sufficient numbers of field mosquitoes for insecticide resistance assessment and residual efficacy tests.

In Thailand, seven Anopheline species (*Anopheles dirus* Peyton & Harrison, *Anopheles minimus* Theobald, *Anopheles maculatus* Theobald, *Anopheles baimaii* Sallum & Peyton, *Anopheles sawadwongponi* Rattanarithikul and Green, *Anopheles aconitus* Dönitz, and *Anopheles psudowillmori* Theobald) are considered vector species of malaria (Tainchum et al. 2014, Tananchai et al. 2019). Due to their higher abundance, *An. dirus*, *An. minimus*, and *An. maculatus* are involved in residual malaria transmission in