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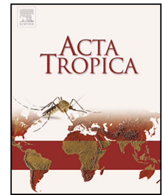

The relationship between wing length, blood meal volume, and fecundity for seven colonies of *Anopheles* species housed at the Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

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ABSTRACT

Established colonies of *Anopheles campestris*, *Anopheles cracens*, *Anopheles dirus*, *Anopheles kleini*, *Anopheles minimus*, *Anopheles sawadwongporni*, and *Anopheles sinensis* are maintained at the Armed Forces Research Institute of Medical Sciences (AFRIMS). Females were provided blood meals on human blood containing citrate as an anticoagulant using an artificial membrane feeder. The mean wing length, used as an estimate of body size, for each species was compared to blood-feeding duration (time), blood meal volume, and numbers of eggs oviposited. Except for *An. campestris* and *An. cracens*, there were significant interspecies differences in wing length. The mean blood meal volumes (mm^3) of *An. kleini* and *An. sinensis* were significantly higher than the other 5 species. For all species, the ratios of unfed females weights/blood meal volumes were similar (range: 0.76–0.88), except for *An. kleini* (1.08) and *An. cracens* (0.52), that were significantly higher and lower, respectively. Adult females were allowed to feed undisturbed for 1, 3, and 5 min intervals before blood feeding was interrupted. Except for *An. campestris* and *An. sawadwongporni*, the number of eggs oviposited were significantly higher for females that fed for 3 min when compared to those that only fed for 1 min. This information is critical to better understand the biology of colonized *Anopheles* spp. and their role in the transmission of malaria parasites as they relate to the relative size of adult females, mean volumes of blood of engorged females for each of the anopheline species, and the effect of blood feeding duration on specific blood meal volumes and fecundity.

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1. Introduction

Malaria is the number one arthropod-borne disease worldwide (Molyneux and Fox, 1993). Female mosquitoes blood feed on warm blooded (mammals and birds) and cold-blooded (reptiles and amphibians) hosts to obtain protein and other nutrients for egg production, while males feed on plant nectar and are not involved in the direct transmission of mosquito-borne pathogens, including malaria (Garnham, 1966; Klein et al., 1987). Blood feeding on human hosts is the link between human – mosquito – *Plasmodium* spp. parasite life cycle. Anautogenous female mosquitoes commonly ingest from 2 to 4 times their mean weights of nutrient starved mosquitoes when fully engorged (Nayar and Sauerman, 1975). In nature, blood feeding is often interrupted by host defensive behaviors, resulting in multiple blood meals on the same or different hosts in order to complete egg development, while at the same time increasing opportunities for infection and multiple transmissions of pathogens (Edman and Kale, 1971; Edman et al., 1974). Blood meal volume also influences mosquito fecundity, as well as the acquisition of pathogens from infected animal and bird hosts to mosquitoes (Kershaw et al., 1955; Woke et al., 1956). Blood meal volume is affected by a range of factors including ambient temperatures, mosquito age, mating status, parity, gonotrophic cycle, blood feeding history (e.g., blood meal host and number of multiple feeds), and infection status (Lehane, 2005). The maturation and number of eggs oviposited is dependent upon the quality (host) and quantity of blood ingested that exceeds the minimum threshold level (Dodd and Burges, 1995). Several authors

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demonstrated a positive correlation between the numbers of eggs oviposited and mean wing length of females for several *Anopheles* species (Shannon and Hadjinicalao, 1941; Briegel, 1990b; Kelly and Edman, 1992; Kitthawee et al., 1992; Hogg et al., 1996; Renshaw et al., 1994). The Armed Forces Research Institute of Medical Sciences (AFRIMS) in Bangkok, Thailand, maintains seven colonies of *Anopheles* spp. in a state of the art insectary. Mosquito colonies are maintained and used for conducting malaria vector competence, malaria transmission blocking, insecticide efficacy and resistance, malaria vaccine trials, and other basic biological studies. These data can be used to better understand the epidemiology of malaria by identifying vector species and potential effects of control measures that target vector populations that more effectively reduce malaria transmission in Thailand and other malaria endemic regions. Six species, *Anopheles dirus, Anopheles minimus, Anopheles campestris, Anopheles cracens, Anopheles sawadwongporni*, and *Anopheles sinensis* are endemic to Thailand and other Southeast Asian countries and associated Pacific Islands (Lee et al., 2007; Obsomer et al., 2007; Foley et al., 2008, 2009; Muenworn et al., 2009; Sinka et al., 2011; Amir et al., 2013). The first established colony of *An. kleini*, recently identified from the Republic of Korea, is a cryptic species (including *An. sinensis* and 3 other *Anopheles* spp.) (Rueda, 2005), and has been implicated as a more competent vector of *Plasmodium vivax* than *An. sinensis* sensu stricto in Korea (Lee et al., 2007; Phasomkusolsil et al., 2014a). *An. sinensis* is commonly collected in Korea, accounting for >90% of all *Anopheles* spp. collected south of Seoul, but also extends to northern Thailand and the Pacific Islands where it is considered a poor malaria vector (Lee et al., 2007; Foley et al., 2009). *Anopheles kleini* has not been detected in Thailand.

Uniform rearing methods are used, based on species requirements, to ensure that colonies approximate reproduction, vector competence, and susceptibility to insecticides in nature. While multiple publications document the relationship of body size, feeding duration, blood meal volume, and egg production for many of the malaria vectors, there is a paucity of information involving the seven species housed at AFRIMS.

The aim of the present study was to determine and compare (1) the relative size of adult females maintained in colony at AFRIMS, (2) the mean volumes of blood consumed by each of the anopheline species, and (3) the effect of blood feeding duration on specific blood meal volumes and fecundity.

2. Materials and methods

2.1. Mosquito colonies and rearing conditions

Female mosquitoes were allowed to feed to repletion for oviposition as part of normal colony maintenance. Approximately 200 eggs were placed on the water surface in plastic trays (size 30 × 35 × 5 cm) containing 1.5 L of distilled water and reared at low larval densities (approximately 150 larvae/pan) and high diet according to methods described by Kitthawee et al. (1992) to produce synchronized emergence and uniformly large adults. Pupae were removed daily, counted, and placed in screen-topped plastic cups (16 cm dia and 16.5 cm high) with 600 ml filtered water and 2–3 cotton balls saturated with a 5% multivitamin solution (Multilim® syrup) placed on the screen top as a food source for newly emerged adults and changed daily. After 2 days, most of the adult mosquitoes had emerged and the water was gently drained from the mosquito cup. Five to seven day-old mosquitoes were provided blood meals via an artificial membrane feeding method as described by Phasomkusolsil et al. (2014b). The vitamin-saturated cotton balls were removed from the holding containers 12 h prior to blood feeding and replaced with water-saturated cotton balls. Six hours prior to blood feeding the water soaked cotton balls were removed to increase blood feeding. Colonies were maintained and all studies conducted at 25 ± 2 °C, 80 ± 10% relative humidity, and 12 h light:12 h dark photoperiod.

2.2. Artificial blood-feeding technique

A circulating water bath LAUDA® (LAUDA DR. R. WOBSER GMBH & CO., KG, Germany), filled and connected to feeding cups via flexible tubing was operated at 37 °C. Sausage casing membranes (pig intestine) were stretched across the bottom of the feeder cups (surface area: 2.54 cm^2), secured with a rubber band, and then the feeder placed in contact with the mesh netting (screened) top of the plastic container holding 5–7 day-old female mosquitoes. Human blood containing citrate as an anticoagulant (obtained from the Thai Red Cross Society) was added (1.5 ml) to the glass feeder well and mosquitoes allowed to blood feed for 30 min. The numbers of fully engorged females were recorded and then provided cotton balls saturated with 5% multivitamin syrup solution as described above. The number of partially blood fed and unfed females were recorded and discarded in accordance with standard protocol.

2.3. Comparison of blood meal volume

Female mosquito body sizes were estimated by measuring their wing length (a standard indicator of body size) (Koella and Lyimo, 1996). One wing was dissected from the body and placed in a drop of distilled water on a microscope slide and, using an eyepiece micrometer, the wing length determined as the distance from the axillary incision to the apical margin (excluding fringes) as described by Nasci (1986).

A total of 15 female mosquitoes of each species that were provided only a 5% multivitamin solution were included as the control group. Cotton balls saturated with 5% multivitamin solution were provided to the control group as previously described and removed 1-hour prior to weighing them individually on an OHAUS® scale (Switzerland). A total of 20 female mosquitoes were allowed to feed uninterrupted for 5 mins, after which the blood source was removed. All unfed and partially fed females were discarded in accordance with standard practice and 15 females selected as the "blood fed" test group. One hour after blood feeding, the females were anesthetized with ether and weight determined as described above. Blood meal weights were calculated as the difference between mean weights of unfed (control) females and individual weights of blood fed females.

2.4. Blood feeding duration

Human blood meal weights and volumes were obtained by varying the lengths of blood feeding time. Females were allowed to feed individually for 1, 3, and 5 min on a membrane feeder as described above. The duration of blood feeding was measured using a stop watch from when the female was observed inserting her mouthparts into the sausage casing membrane and terminated when the female withdrew her proboscis. Females that did not initiate blood feeding within five minutes or completed blood feeding before the predetermined feeding period were discarded and data were excluded from the study. Ten females that fed for the allotted times were anesthetized with ether and weighed 1-hour after blood feeding and the quantity (weight) of blood ingested by each individual was calculated by subtracting the mean weights of the unfed (control group) from individual blood fed females as described above. Blood volume was determined as: $V = M/D$, where V = blood volume (mm^3), M = blood weight (mg), and D = density (1.05 mg/mm^3) (Jeffery, 1956). Since the anopheline species used in this study did not mate in the cages under laboratory conditions, they were mated manually as described by Ow Yang et al. (1963). A total

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