Comparative Studies on the Morphometry of Male Genitalia and Frequency of Clasper Movements During Induced Copulation of *Anopheles balabacensis* (Perlis Form) and *Anopheles dirus* (Bangkok Colony Strain)

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ABSTRACT: Comparative studies on the morphometry of male genitalia and the frequency of clasper movements during induced copulation were done in the laboratory with colony strains of the natural mating *Anopheles balabacensis* (Perlis Form) and the induced mating *Anopheles dirus* (Bangkok Colony Strain). In the Perlis Form the width and the length of phallosome, coxite, style and coxite plus style were significantly larger than in *dirus* on most particulars. The time of clasper movements, mating time and frequency of clasper movements in the Perlis Form were also significantly less than in *dirus*. The results suggested that these two measurements can be used for differentiation of these two species. The mating behavior of stenogamy in the Perlis Form and eurygamy in *dirus* were also discussed.

INTRODUCTION

The leucosphyrus species group of *Anopheles (Cellia)* includes *balabacensis* Baisas, *b. baisasi* Colless, *b. introlatus* Colless, *dirus* Peyton & Harrison, *elegans* James, *hackeri* Edwards, *cristatus* King & Baisas, *pujutensis* Colless, *riparis* King & Baisas, *r. macarthuri* Colless, *takasagoensis* Morishita, *leucosphyrus* Donitz and *sulawesi* Koesoemawinangoen. In the Southeast Asian and Indian sub-regions of the Orient some species of the group are of major public health significance. At least two species, i.e., *leucosphyrus* and *balabacensis* sensu stricto are recognized as primary vectors of human malarial parasites (Reid 1968). Peyton and Harrison (1979) described the species previously called *balabacensis* in Thailand as a new species, *dirus*. This species is morphologically distinct in the adult, pupal and fourth larval stages from topotypic *balabacensis* from Balabac Island, Philippines, and certain other areas of the Philippines and Malaysia. Kanda et al. (1980) also reported on laboratory strain of induced mating *An. dirus* from Perlis State Malaysia by observation on the adult and pupal characters. This *dirus* had pupal characters which differed from topotypic *balabacensis* and *dirus* as described by Peyton and Harrison (1979), but Kanda et al. (1980) tentatively treated it as *dirus* due to the characters of the adult stage. Recently, Baimai et al. (1981)

described the karyotype differentiation in laboratory strains of natural mating *balabacensis* (Perlis Form) and induced mating with *dirus* (Bangkok Colony Strain) and also *An. takasagoensis*. The results showed that each of these three taxa represents a full biological species. Furthermore, they support the recent description of the new species, *dirus*, which replaced the species previously considered to be *balabacensis* in Thailand (Peyton and Harrison 1979). In view of the importance of *balabacensis* s.s. and *dirus*, we feel these species and colony strains need to be defined more thoroughly. Accordingly, the morphometry of male genitalia and frequency of clasper movements during induced copulation of the laboratory strains of natural mating *balabacensis* (Perlis Form) and induced mating *dirus* (Bangkok Colony Strain) are described below to be used as additional characters for differentiation of these two species.

MATERIALS AND METHODS

Mosquito strains

The laboratory strain of natural mating *An. balabacensis* (Perlis Form) and induced mating *An. dirus* (Bangkok Colony Strain), the same strains as studied by Baimai et al. (1981) were obtained from the Armed Forces Research Institute of Medical Sciences (AFRIMS) laboratory, Bangkok, and maintained for many generations in the insectary of the Department of Medical Entomology, Faculty of Tropical Medicine, Bangkok, since 1979. For *dirus*, the colony was maintained by induced mating technique described by Ow Yang et al. (1963). Attempts were made to start a natural mating colony of *dirus* by gathering the newly emerged males and females in a 30 x 30 x 30 cm cage (300 males : 150 females) but they proved unsuccessful. No oviposition and insemination rates were obtained (0/138). Whereas, *balabacensis* (Perlis Form), under the same conditions, yielded oviposition, insemination and hatchability rates of 60.8% (76/125), 67.2% (84/125) and 90.43% (7835/8664), respectively. Known morphological differences between *dirus* and *balabacensis* s.s. in the adult and pupal stages are the development of the accessory sector pale (Asp) spot on the costa of the adult wing and the lengths of pupal seta 9-III-V, with 9-IV usually being expressed as long or short. As reported by Peyton and Harrison (1979) in feral *dirus* (from Thailand), there is never an Asp on the costa and the length and ratios of pupal seta 9-III-V were, 9-IV 0.030-0.059 mm (0.043 mm), ratios of length of IV/III 1.50-3.19 (2.14) and IV/V 0.28-0.53 (0.41). In topotypic *balabacensis* from Balabac Island, Philippines, 73% had Asp present on the costa on one or both wings the length of pupal seta 9-IV 0.056-0.089 mm (0.074 mm) and the ratios of length of IV/III 3.05-5.54 (4.11) and IV/V 0.65-1.05 (0.81). Our observations on 20 females and 20 pupal skins of *balabacensis* (Perlis Form), revealed the Asp was not present (0%*) on the costa on one or both wings and the length of pupal seta 9-IV 0.056-0.097 mm (0.079 mm), ratios of length IV/III 3.48-7.08 (4.67) and IV/V 0.59-0.88 (0.79). The former adult character was not different from *dirus* (Bangkok Colony Strain), while the pupal characters of *balabacensis* (Perlis Form) were in the normal ranges of topotypic *balabacensis* from Balabac Island, Philippines.

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*Confirmed by Mrs. Rampa Rattanarithikul, AFRIMS, Bangkok.

Preparation and measurement of male genitalia

Male genitalia were prepared by cutting off the last abdominal segments of adult males, aged 5 days, slide-mounting each in Gater's medium in an anatomically ventral position as described by Reid (1968), and leaving it for three days until the exoskeleton was clear. The specimens were assessed by measuring the widths and lengths under a compound microscope using the camera lucida at various sites as detail descriptions in Figure 1. Thirty genitalia were used from each species.

Clasper movement of male genitalia during induced copulation

In this study 30 individuals of 5-day old males were examined, from each of the two species. During induced copulation the females are first clasped with the claspers of the males, remain coupled for a long period before the initiation of the pumping motion with movements of their claspers (both coxites and styles) which continues until later separated. The mating time and frequency of clasper movements were counted using electric watch and binocular microscope. The experiments were performed at room temperature (25 ± 1° C).

RESULTS

The width and length of phallosome, coxite, style and coxite + style of *balabacensis* (Perlis Form) were significantly larger than those of *dirus*, t-distribution of all tests; degrees of freedom = 58, $p < 0.001$, except in left style $p < 0.005$ (Table 1). The mean widths and lengths of phallosome were 87.43 ± 8.12 μ and 79.65 ± 10.59 μ, coxite 105.04 ± 8.90 μ (Right), 104.94 ± 8.67 μ (Left) and 279.5 ± 26.68 μ (Right), 285.6 ± 26.21 μ (Left). The lengths of style and coxite + style were 366.17 ± 17.85 μ (Right), 341.2 ± 23.37 μ (Left) and 643.67 ± 31.06 μ (Right), 627.00 ± 39.23 μ (Left). Whereas in *dirus* the mean widths and lengths of phallosome were 79.23 ± 4.70 μ and 67.86 ± 10.01 μ, coxite 97.09 ± 8.60 μ (Right), 93.76 ± 7.88 μ (Left) and 258.43 ± 13.50 μ (Right), 258.80 ± 11.91 μ (Left). The lengths of style and coxite + style were 330.73 ± 23.10 μ (Right), 324.57 ± 22.49 μ (Left) and 589.17 ± 28.81 μ (Right), 583.37 ± 27.29 μ (Left). The time of pre-clasper movements, clasper movements, mating time and frequency of clasper movements during induced copulation in *balabacensis* (Perlis Form) and *dirus* (Table 2) were 9 ± 1.69 sec, 11.03 ± 2.37 sec, 20.03 ± 3.17 sec, 21.47 ± 6.32 and 8.93 ± 0.85 sec, 14.13 ± 2.16 sec, 23.07 ± 2.17 sec, 35.5 ± 5.16, respectively. Only the time of clasper movements, mating time and frequency of clasper movements of both species were statistically different, t-distribution of all tests, degrees of freedom = 58, $p < 0.001$.

DISCUSSION

Investigations of the morphology of adult, pupal and fourth larval stage, crossmating experiments and cytogenetic studies have so far been used for the comparative studies of *leucosphyrus* species group. The most advanced and

detailed investigations of this species group have been carried out on species or geographic forms of the Balabacensis Species Complex which morphologically are highly variable. Recently Peyton and Harrison (1979) described the species previously called *balabacensis* in Thailand as a new species, *dirus*. This species is morphologically distinct in the adult, pupal and fourth larval stage from topotypic *balabacensis* from Balabac Island, Philippines. Our studies were designed to define these two species based on the morphometry of male genitalia and frequency of clasper movements during induced copulation. The results of the studies revealed that the width and length of male genitalia of *balabacensis* (Perlis Form) were significantly larger than *dirus* (Bangkok Colony Strain) (Table 1). The time of clasper movements, mating time and frequency of clasper movements in *balabacensis* (Perlis Form) were significantly less than *dirus* (Bangkok Colony Strain) (Table 2). As determined by our studies, it could be concluded that these characters might be used as diagnostic tools for these two species, particularly the frequency of clasper movements during induced copulation. Kreutzer and Kitzmiller (1971) found certain behavioral differences associated with copulation in *Anopheles punctipennis* species group, i.e., *An. punctipennis* Say and *An. perplexens* Ludlow. The pumping motion associated with clasper movements is of short duration in *punctipennis* as compared with *perplexens* in which it continues for a longer period of time. Kanda and Oguma (1976) also reported that the frequency of clasper movement can be used to differentiate the various strains of *An. sinensis* (Japan Strain) which morphologically were highly variable. Therefore, according to the evidence mentioned above, the frequency of clasper movements is a common and useful diagnostic character of the species group. It is never influenced by rearing conditions, age, generation or other parameters. It also does not change when the male copulates with a female of other species (Kanda and Oguma 1976).

Another distinct point in our observations on these two species was the difference in mating behavior, stenogamy in *balabacensis* (Perlis Form) and eurygamy in *dirus* (Bangkok Colony Strain). It was recently shown that behavioral polymorphism of stenogamy and eurygamy are inherited and is obviously controlled by one or more genes located on the Y chromosome (Fracarro et al. 1977). To our results, this difference in mating behavior might also be added as another means of differentiating *balabacensis* (Perlis Form) and *dirus*. The genitalia of the Perlis Form is larger than that of *dirus* and the former has a shorter duration of clasper movements and mating time than the latter species. Therefore, the male genitalia of *balabacensis* (Perlis Form) seems to be highly effective in mating, which might influence the success in copulation in a small cage.

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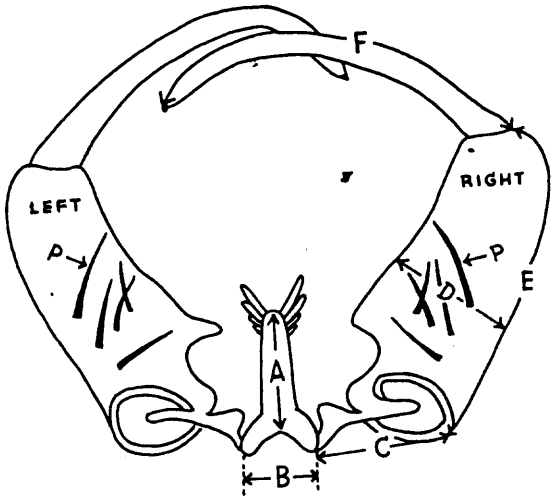

FIGURE I. Schematic measurement of the ventral view of male genitalia at various sites. A: length of phallosome, B: width of phallosome, C: length between base of phallosome and origin of coxite, D: width of coxite at the origin of last parabasal spine (P), E: length of coxite, and F: length of style.

TABLE 1. Comparative morphometry of the male genitalia of *An. balabacensis* (Perlis Form) with *An. dirus* (Bangkok Colony Strain)

Mosquito species*	The length and width in micron of male genitalia at various sites (range)							
	A	B		C	D	E	F	E + F
An. balabacensis (Perlis Form)	79.65±10.59 (58.97-99.99)	87.43±8.12 (64.10-110.25)	Right:	93.42±6.54 (82.05-110.25)	105.04±8.90 (89.74-125.64)	279.50±26.68 (239-375)	366.17±17.85 (330-405)	643.67±31.06 (575-715)
			Left:	93.84±6.54 (82.05-110.25)	104.94±8.67 (94.87-125.64)	285.60±26.21 (250-380)	341.20±23.37 (300-400)	627.00±39.23 (550-740)
An. dirus (Bangkok Colony Strain)	67.86±10.01 (51.28-84.61	79.23±4.70 (71.79-92.30)	Right:	87.67±3.62 (82.05-94.87)	97.09±8.60 (79.48-115.38)	258.43±13.50 (235-300)	330.73±23.10 (260-380)	589.17±28.81 (497-655)
			Left:	88.12±6.01 (76.92-107.69)	93.76±7.88 (79.48-107.69)	258.80±11.91 (235-285)	324.57±22.49 (265-360)	583.37±27.29 (515-645)

*Thirty mosquitoes for each experiment

TABLE 2. Comparative measurement of mating time in second and frequency of clasper movements per copulation of *An. balabacensis* (Perlis Form) with *An. dirus* (Bangkok Colony Strain).

Mosquito* species	Time of pre-clasper movements (range)	Time of clasper movements (range)	Frequency of clasper movements (range)	Mating time (range)
An. balabacensis (Perlis Form)	9 ± 1.69 (6-12)	11.03 ± 2.37 (7-17)	21.47 ± 6.32 (8-34)	20.03 ± 3.17 (15-26)
An. dirus (Bangkok Colony Strain)	8.93 ± 0.85 (8-11)	14.13 ± 2.16 (11-21)	35.50 ± 5.16 (28-52)	23.07 ± 2.17 (20-30)

*Thirty mosquitoes for each experiment