mesanepimeral setae) if the basal pale band of the hindtarsomere covered ≥0.3% of the segment. To our knowledge, Belkin et al. (1970) is the only reference to discuss variation in the size of the BP. These authors state that in some populations, the BP shows considerable variation and is sometimes incomplete dorsally or nearly absent. The detailed analysis of variation described in this study should facilitate the utilization of this key diagnostic character by demonstrating that although almost 98% of the specimens examined did fit the key, some specimens could not be identified.

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TIMING OF PUPAL-ADULT APOLYSIS IN MOSQUITOES[1]

E. D. WALKER[2] AND W. S. ROMOSER[3]

Apolysis, the separation of cuticle and epidermis prior to new cuticle formation, is a key, morphologically evident event which signals the initiation of molting (Hinton 1973). Despite the developmental significance of this event, it has been little studied in mosquitoes (Walker and Romoser 1982). The purpose of this study was to determine the timing of pupal-adult apolysis in *Aedes aegypti* (Linn.), *Ae. sollicitans* (Walker), *Culex nigripalpus* Theobald, and *Psorophora columbiae* (Dyar and Knab).

Eggs of *Ae. sollicitans, Cx. nigripalpus* and *Ps. columbiae* were obtained from natural populations in the vicinity of the Florida Medical Entomology Laboratory, Vero Beach, Florida. Larvae were reared at 27±1°C. and fed brewer's yeast and liver powder *ad lib.* Pupae of known hourly ages (± 1 hr) postpupation were killed and fixed in hot alcoholic Bouin's solution, dehydrated and infiltrated with paraplast, cut into 7.0 μm serial sagittal sections, mounted on slides with Mayer's albumin, and stained according to the modified azan trichrome technique (Hubschman 1962). Specimens of *Ae. aegypti* Rockefeller strain were prepared similarly, except that a dioxane-paraffin dehydration and infiltration method was used, and the slides were stained with Masson's triple stain[4]. Specimens of ages spanning the entire pupal stadium of each species were prepared.

Four integument sites were examined, at 1000X with a light microscope, on the midsagittal section of each specimen: (1) scutum, (2) third abdominal tergum, (3) seventh abdominal sternum, and (4) anterior wall of ventral air space. These different sites were examined to take into account variation in timing of apolysis about the pupal body (Walker and Romoser 1982).

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[4] Lane, J. E. 1963. The development of the oesophageal diverticula in the yellow fever mosquito, *Aedes aegypti* (L.) (Diptera: Culicidae). Ph.D. Dissertation, The Ohio State University, Columbus, OH, 99 pp.

Data regarding duration of pupal stages (measured from pupation to adult emergence) of *Ae. sollicitans, Cx. nigripalpus* and *Ps. columbiae* are from Nayar (1968) and Nayar and Sauerman (1970). The specimens of these three species used in our study were from the same natural population as those studied by Nayar and Sauerman. Pupal stage duration in *Ae. aegypti* was determined by placing newly emerged pupae individually in vials and holding them to adult emergence.

Since pupal cuticle and adult epidermis often become separated during fixing or sectioning, we used the time of initial molting fluid secretion as an indication of recent apolysis. Molting fluid is readily observable as a granular exudate in the exuvial space (Romoser and Walker 1982). Secretion of molting fluid is known to occur simultaneously with apolysis in other insects (Passonneau and Williams 1953).

Table 1 shows the timing of molting fluid secretion (i.e. recent pupal-adult apolysis) and duration of the pupal stage in each of the four species. In each species, apolysis occurred first at the scutum and 1–2 hr later at the other sites. In all cases apolysis occurred early, in general before 20% of the pupal stage was completed. Thus most of the time between pupation and adult emergence in mosquitoes is spent as a pharate adult and not a pupa *per se*, as shown by Hinton (1973) in other Nematocera.

raised at 27°C. Gnatzy (1969) observed that in *Cx. pipiens* Linn. reared at 26°C, pupal-adult apolysis occurred at 15 hr in the 44-hr pupal stage.

Knowledge of the timing of pupal-adult apolysis may be useful in studies of physiological effects of growth disrupting insecticides or any physiological or developmental studies which require delineation of pupae from pharate adults.

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Table 1. Age at pupal-adult apolysis and duration of pupal stadium.[1]

Species	Time of apolysis (hours)	No. of specimens showing apolysis	No. of specimens examined[2]	Mean duration of pupal stadium
Aedes aegypti	6–8	12	78	50.0[3]
Ae. sollicitans	6–7	9	99	39.0[4]
Culex nigripalpus	5–6	8	127	34.5[5]
Psorophora columbiae	6[6]	2	68	31.0[4]

[1] Based on results from all study sites.
[2] Specimens examined represented entire pupal stadium.
[3] Range = 45–55; n = 38.
[4] From Nayar and Sauerman (1970).
[5] From Nayar (1968).
[6] 5-hour specimens were not available; apolysis had not yet occurred in the 4-hour specimens (n = 8) examined.

Other researchers have noted early occurrence of pupal-adult apolysis in mosquitoes. Christophers (1960, p. 359) observed ". . . early retraction of the hypoderm. . ." from the pupal cuticle covering the developing mouthparts of *Ae. aegypti*, and Clements (1963, p. 90) generalized that in mosquitoes "A few hours after pupation the epidermis of the mouthparts retracts from the pupal cuticle . . .". Walker and Romoser (1982) found that pupal-adult apolysis occurred early (10–12 hr postpupation) in the 60-hr pupal stadium of *Ae. triseriatus* (Say)

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