THE TIMING OF DIURESIS IN NEWLY-EMERGED ADULTS OF *CULICOIDES MELLEUS* AND *CULICOIDES VARIIPENNIS* (DIPTERA: CERATOPOGONIDAE)[1]

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ABSTRACT. Diuresis in the first 20 min after emergence was examined in males and females of *Culicoides melleus* and *Culicoides variipennis*. Both sexes of both species excreted most fluid (estimated as drops per minute) in the period between 2 and 8 min after emergence. *Culicoides melleus* adults, however, were more productive than *C. variipennis*. Males of both species excreted significantly more drops of fluid than females within the 20 min period of observation. Measurement of the sizes of drops excreted by *C. variipennis* showed that the largest drops were eliminated immediately after emergence.

INTRODUCTION

The processes of excretion in mosquitoes have been described in some detail, both with respect to events occurring immediately after ecdysis (Venard and Guptavanij 1966, DeGuire and Fraenkel 1973, Jones and Brandt 1981) and after ingestion of blood (Boorman 1960, Stobbart 1977). Biting midges appear to resemble mosquitoes in many of their excretory functions, but midges have never been examined specifically. During studies of the sexual behavior of *Culicoides melleus* (Coq.) (Linley and Adams 1972), it was seen that the ability of *C. melleus* males to achieve copulation very shortly after emergence depended upon prior elimination of fluid through the anus. Males were otherwise unable to bend their abdomens ventrally to achieve the copulatory position (Linley and Adams 1972). Since newly-emerged *C. melleus* and also *Culicoides variipennis* (Coq.) adults were readily obtainable, opportunity was taken to study the time-course of excretion in the first 20 min of adult life. No attempt was made to determine the origin of the excreted fluid, or its chemical composition. Terms such as urine, meconium, or others implying origin in different parts of the gut have therefore been avoided. Jones and Brandt (1981) suggest the general term cloacal fluid, and this has been applied here.

MATERIALS AND METHODS

Male and female *Culicoides melleus* were collected as pupae from the natural habitats, separated by sex on return to the laboratory and kept until needed at 16°C in small petri dishes containing wet sand. Adults could then, after a few days, be induced to emerge at will by placing the petri dishes on a warm (34°C) surface (Linley and Adams 1972). Keeping the sexes separate was necessary to prevent copulation within a few minutes of emergence. Newly emerged laboratory colony *C. variipennis* were obtained in a similar way from groups of pupae kept in the cool incubator on damp filter paper.

As each adult insect freed itself from the pupal skin it was allowed to crawl onto a fine artist's brush, from which it was quickly dislodged into a circular, Plexiglas® observation cell (9.5 mm wide, 3.2 mm deep), covered with a coverglass (Linley and Carlson 1983) and placed under a stereo-microscope. Emission of individual drops of fluid was recorded continuously on an event recorder (Evans 1975), until 20 min had elapsed from the time of emergence. Recordings were obtained from 20 individuals for each sex and species.

To obtain an index of the relative sizes of excreted drops, drops from 5 *C. variipennis* males and 5 females were collected sequentially and as nearly as possible in rows, by catching them on thoroughly cleaned microscope slides held beneath individuals tethered at emergence to minuten nadeln. Drops collected in this way dried to form circular impressions (DeGuire and Fraenkel 1973), the diameters of which were measured with an ocular micrometer.

RESULTS

The time-course of excretion by 1 min increments after emergence is shown for both *C. melleus* and *C. variipennis* in Fig. 1. The timing of diuresis was similar in the two species. The number of droplets of cloacal fluid increased very rapidly from hardly any in the first minute after emergence to a maximum during the third minute, followed by a steady decline in each case. *Culicoides melleus* adults of both sexes excreted considerably more drops of fluid than *C. variipennis* (Fig. 1), a rather surprising result because *C. melleus* is the smaller of the two species. A comparison of the total number of drops eliminated in the first 20 min (Table 1) again

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Table 1. Comparison of total drops of cloacal fluid secreted by *Culicoides melleus* and *Culicoides variipennis* males and females in the first 20 minutes after emergence.

Species	Mean total drops secreted (± SE) ♂	♀	Difference	*t*
Culicoides melleus	33.9 ± 2.1	26.3 ± 1.9	7.6	2.656††
Culicoides variipennis	16.6 ± 1.3	10.3 ± 0.7	6.3	4.269†††

†† $P < 0.02$, ††† $P < 0.001$.

indicated the degree to which *C. melleus* of both sexes were more productive than *C. variipennis*, but also that males of each species excreted significantly more drops than females. The ratio (♂:♀) was somewhat lower for *C. variipennis* (1.28:1) than in *C. melleus* (1.58:1).

As the drops of fluid were collected on slides, for subsequent measurement, no record was kept of the time elapsed since emergence. Drop size was therefore related to the order of excretion (i.e., drop number). An approximate relationship to time could be derived from consideration of the cumulative total excreted (from Fig. 1), but little useful information could be gained from this procedure. By preliminary analysis of variance, the regression model y = a + b log× best described the raw data for both males and females. To produce a linear relationship and simplify analysis, therefore, a logarithmic transformation was used to examine the regression of mean drop diameter for each sex on drop number (Fig. 2). There was a significant regression for both males and females (Fig. 2), indicating that larger drops of fluid were excreted immediately after emergence and that drop size decreased with order (and time), at least within the period measured. There was no significant difference between the regression coefficients for the two sexes. Mean drop diameters were, however, greater for females than for males for 12 of the first 14 drops secreted (Fig. 2), suggesting that although females excrete fewer drops than males (Table 1), the drops tend to be larger.

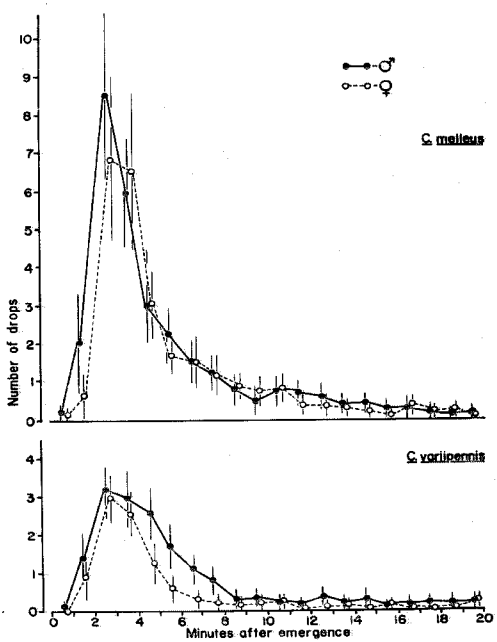

Fig. 1. Time-course of excretion of cloacal fluid by male and female *Culicoides melleus* and *C. variipennis* in the first 20 min after emergence. Points and vertical lines show means and 95% confidence intervals, respectively.

DISCUSSION

Not surprisingly, the pattern of diuresis following emergence in midges seems to conform generally with observations in mosquitoes, *Aedes aegypti* (L.) having been studied particularly closely (DeGuire and Fraenkel 1973, Jones and Brandt 1981, Gillett 1983). Gillett measured the volume of fluid excreted and obtained mean values for the rate of elimination in the first 40

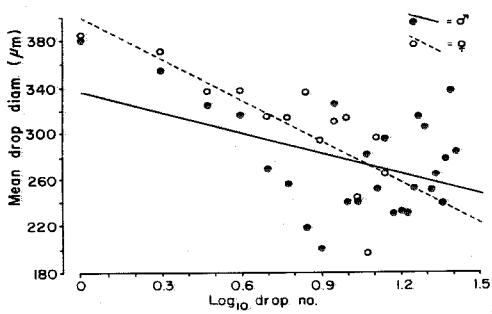

Fig. 2. Regression of mean drop diameter on drop number ($\log_{10}$ transformed) excreted by male and female *Culicoides variipennis*. For males, be = −62.9 ($P < 0.02$); for females, b = −122.7 ($P < 0.001$).

min after emergence. His data for 0–10 min show a decrease in the rate for both males and females (the rate in males being somewhat less than in females), while during the same period, Jones and Brandt (1981) counted almost identical numbers of drops excreted by 20 individuals (13 males, 7 females) for the periods 0–5 min and 5–10 min. This suggests, as noted also by DeGuire and Fraenkel (1973), that drop size decreases with time in the period immediately after emergence. Midges conform with this pattern (Fig. 2), the logarithmic fit in the untransformed data suggesting that the decrease in drop size is especially rapid when diuresis first starts. Jones and Brandt (1981) tabulated the numbers of drops for individual mosquitoes, by 5 min increments up to 1 hr. A statistical examination of their results, however, shows no significant difference between the sexes in mean total drop number, in contrast to the situation in midges (Table 1).

When adult *Culicoides* first clamber out of the pupal skin they are quite distended. The insects are able to walk, but sustained flight is impossible. Exposure to predation may be especially important at this time and, to that extent, selective pressure probably operates to maintain populations in which elimination of cloacal fluid occurs very soon after emergence. For the male *C. melleus*, about whose sexual activity and physiology a good deal is known (Linley and Adams 1972, Linley and Mook 1975, Hinds and Linley 1974), additional selective pressures probably exist. Much evidence, summarized briefly by Linley (1975) suggests that mating in *C. melleus* occurs very early in adult life. Males are fully potent in the first hour of their lives (Hinds and Linley 1974) and virgin females are most receptive when very young (Linley and Adams 1974). Copulation probably begins as the newly emerged insects encounter one another on the surface of the breeding sites. However, the precopulatory maneuvers of the male require him to bend his abdomen sharply in the ventral direction (Linley 1984), and he cannot do this until excretion reduces the degree of abdominal distension. Males are, however, capable of copulation within a few minutes of emergence (once fluid is eliminated) and are able at this time to transfer a full complement of sperm (Hinds and Linley 1974). The newly-emerged male, therefore, enters a situation of immediate competition for a mate, enhanced by the fact that males usually outnumber females (Linley and Mook 1978). A prompt diuresis ensures that a male is quickly ready to achieve copulation if the opportunity arises.

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