TEMPERATURE EFFECTS ON THE GONOTROPHIC CYCLE OF *CULICOIDES VARIIPENNIS* (DIPTERA: CERATOPOGONIDAE)

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ABSTRACT. The duration of oogenesis and time to oviposition after blood feeding was studied in *Culicoides variipennis* held at constant laboratory temperatures. As assessed by daily dissection of ovaries, the time required for ≥80% of females to complete egg development at 13, 17, 21, 27, 30 and 34°C was 10, 5, 4, 3, 2 and 2 days, respectively. Mean times to oviposition at these temperatures were 13.8, 8.2, 4.9, 3.6, 3.0 and 2.6 days, while modal values were 13, 6, 4, 3, 3 and 3 days, respectively. Allowing 7 days after onset of oviposition in groups of 100–150 females, cumulative eggs laid/live female were 4.4, 62.6, 68.9, 63.5, 66.5 and 62.4, respectively. These data should prove helpful in estimating the duration of the gonotrophic cycle and deriving daily survivorship estimates from seasonal parity studies in the field.

INTRODUCTION

Vectorial capacity (C) provides an estimate of the relative capability of an arthropod population to transmit a disease agent within a population of vertebrate hosts. The concept originated for mosquito vectors of malaria (Garrett-Jones 1964), but also is relevant for vectors of arboviruses. With the addition of a term to account for variable vector competence (Reisen 1989), vectorial capacity is defined as follows:

$$C = ma^2Vp^n/(-\log_e p),$$

where C = number of new infections per case per day, m = number of vectors per host, a = number of blood meals taken by a vector per host per day, V = vector competence (physiological susceptibility of the vector to pathogen replication), p = daily survival probability of the vector, and n = number of days that pass between pathogen acquisition by the vector and the time it becomes capable of infecting a new vertebrate host (extrinsic incubation period).

The biting midge *Culicoides variipennis* (Coquillett) is regarded as the primary vector of bluetongue virus (BLU) to North American ruminants (Jones et al. 1981) and is, to our knowledge, an anautogenous species. Stage-specific survivorship estimates for this species have been obtained from field parity data, utilizing either parous pigment or changes in abdominal tergite patterns to categorize females by external examination (Linley and Braverman 1986). Tentative estimates of daily survivorship have been derived from these parity rates (Mullens and Rutz 1984, Mullens 1985, Linhares and Anderson 1989), based on a gonotrophic cycle duration of approximately 4 days at a constant 22°C (Mullens and Schmidtmann 1982). Mullens and Rutz (1984), working with *C. variipennis* populations in New York State, proposed that this period perhaps was unrealistically brief in many field settings. Daily survivorship and duration of the gonotrophic cycle also may be estimated mathematically from time series analysis of parity data (Birley and Boorman 1982, Braverman et al. 1985) or directly from mark-release-recapture studies, sometimes coupled with gonotrophic age assessments, in the field (e.g., Reisen et al. 1981).

Variability in the duration of the gonotrophic cycle has implications for calculation of feeding frequency (and thus biting rates) as well as calculation of daily survivorship rates from parity data. Linley (1965, 1966) demonstrated that temperature could markedly affect the duration of the gonotrophic cycle in blood-feeding Ceratopogonidae. The present study was designed to determine the effects of different constant temperatures on oogenesis and oviposition in *C. variipennis* over time.

MATERIALS AND METHODS

Female *C. variipennis sonorensis* Wirth and Jones (AK strain, originally from southern Idaho) were obtained from colonies maintained according to the modified methods of Jones et al. (1969). Flies (1–2 days old) were fed a meal of defibrinated sheep blood through a silicone membrane (Hunt and McKinnon 1990). On the day of feeding they were anesthetized with CO_2 and sorted on a chill table into groups, including 10% males to increase mating opportunities. Flies were held in 237 ml cardboard containers,

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and a wick with 10% sucrose was provided through a hole in the side of the container. A small cup with a moist pad and filter paper disk was continuously available for oviposition.

Flies were held at constant temperatures of 13 ± 1, 17 ± 0.5, 21 ± 2, 27 ± 1, 30 ± 1 and 34 ± 1°C. Temperatures were monitored with recording thermographs during the trial. One hundred fully engorged females (as judged by external examination) were used for all temperatures except 13 and 34°C, for which 150 engorged females were used. Flies were checked at 24 h intervals, and dead flies were noted and removed. Five live females were removed each day for dissection of the ovaries to note the stage of oocyte development (Linley 1965, Mullens and Schmidtmann 1982). Ten primary follicles were scored/female by examination under a compound microscope. Dissection ceased when all 5 females from a given temperature were fully gravid or had already oviposited. The oviposition pad was removed each day for counting of eggs and a new one provided; the wick also was examined for eggs and replaced as necessary. The daily egg counts were divided by females alive on that day to obtain a measure of egg deposition/female/day. Weighted mean times of egg deposition were calculated for each temperature from the daily totals according to the formula

$$T_{mean} = \Sigma ed/\Sigma e_i,$$

where T_{mean} = weighted mean time of egg deposition, e = number of eggs deposited on a given day, d = days after feeding on blood, and Σe_i = sum of all eggs deposited over the entire egg collection interval from day 1-n.

RESULTS

Oogenesis proceeded more rapidly at higher temperatures. The first mature eggs (stage 5) were seen at 2 days at 34 and 30°C, at 3 days at 27 and 21°C, 4 days at 17°C, and 9 days at 13°C. Days required for at least 4 of 5 females to mature eggs ranged from 2 days at 30 and 34°C to 10 days at 13°C. Mean oocyte development scores are presented in Fig. 1. There was general gonotrophic harmony (synchrony in oocyte development) within individual females at all temperatures. Resorption of primary oocytes in a few ovarioles was evident in nearly all females by stage 3 of oocyte development; marked resorption (> approximately 30%) was noted only in 2 females held at 13°C and one held at 21°C. There was some variability among females within a temperature, though standard errors for the oocyte development scores still were <20% of the means by day and temperature.

Oocyte developmental rate (the reciprocal of the days required for at least 80% of the females at a given temperature to mature eggs) is presented in Fig. 2. A linear regression described 94.7% of the variability in the data set.

The time pattern for oviposition also depended on temperature (Fig. 3). At 30 and 34°C

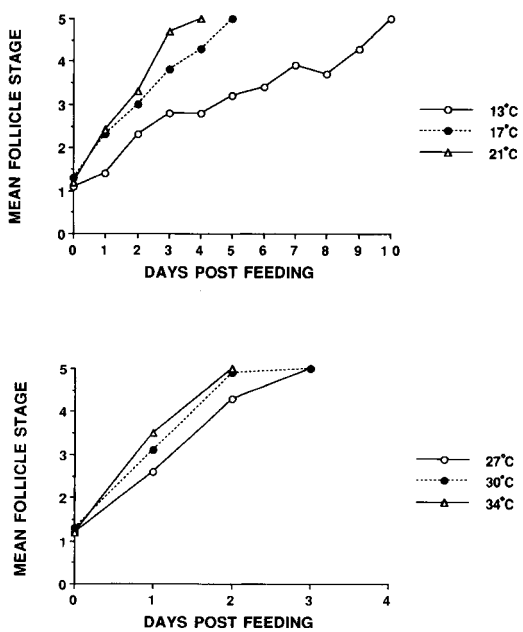

Fig. 1. Oocyte development (mean of 5 females/day) following a blood meal in *Culicoides variipennis* held at constant temperatures in the laboratory (stage 5 = fully mature egg).

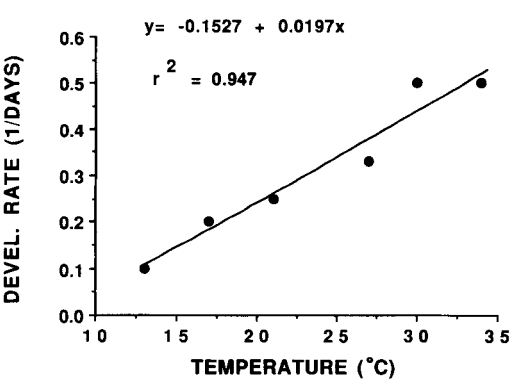

Fig. 2. Relationship between temperature and time needed for ≥ 80% oocyte maturation in *Culicoides variipennis*.

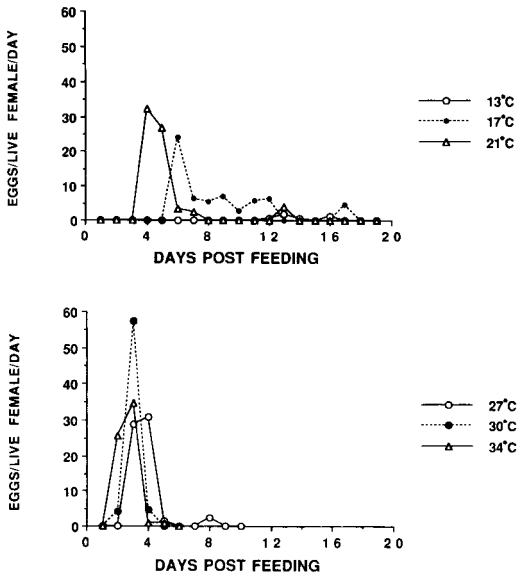

Fig. 3. Frequency distribution (days after blood feeding) of daily oviposition by *Culicoides variipennis* at constant laboratory temperatures.

some eggs already had been deposited by 2 days after blood feeding. The first egg deposition was noted at 3 days at 27°C, 4 days at 21°C, 6 days at 17°C and 13 days at 13°C. Oviposition was quite synchronous for females held at temperatures ≥ 21°C, with nearly all the eggs deposited over a 1–3 day period. In contrast, oviposition extended over a week's time at 17°C, and very few females oviposited at all at 13°C. At temperatures of 13, 17, 21, 27, 30 and 34°C, mean/modal times of egg deposition were 13.8/13, 8.2/6, 4.9/4, 3.6/3, 3.0/3 and 2.6/3 days, respectively. Cumulative egg totals/live female (over a 7 day period after onset of oviposition) were 4.4, 62.5, 68.9, 63.5, 66.5 and 62.4 eggs/female, respectively. The relationship between temperature and mean time to oviposition is shown in Fig. 4. The regression described 99.8% of the variability in the data set.

DISCUSSION

As expected, lower temperatures substantially prolonged both oocyte development and subsequent egg deposition. Consequently, the variance around mean time to oviposition was greater at the lower temperatures. While this to a certain extent compromises the validity of the regression equation, it still should be very useful in generating estimates of time to oviposition based on daily temperatures. The relationships between oocyte developmental times and temperature in *C. variipennis* are similar to those noted by Linley (1965, 1966) for *Culicoides barbosai* Wirth and Blanton, *C. furens* (Poey) and *Leptoconops bequaerti* (Kieffer). Developmental time at 21°C was essentially identical to New York *C. variipennis variipennis* fed human blood and held at 22°C (Mullens and Schmidtmann 1982). Definition of time to oocyte maturation and deposition at 30 and 34°C would have been improved by more frequent examination, but these differences likely would have been of minor practical significance.

This study confirms that some degree of oosorption apparently is normal in *C. variipennis* after a blood meal. The phenomenon also commonly occurs in other blood-feeding ceratopogonids (Linley 1965, 1966), and is critical for successful gonotrophic aging by examination of degenerative follicular relics in *C. variipennis* (Mullens and Schmidtmann 1982). While Linley (1965) noted increased resorption in *L. bequaerti* held at 37°C, we did not see increased resorption in *C. variipennis* at the highest temperature used in this study (34°C).

It would be desirable to perform similar studies of the gonotrophic cycle at variable daily temperatures, though such facilities are less commonly available compared with those maintaining constant temperature. However, for many biological phenomena, average daily temperatures (e.g., [maximum + minimum]/2) are suitable for calculation of rates (Baskerville and Emin 1969), at least provided daily temperatures are between the lower and upper developmental thresholds. Almost nothing is known of selection of resting sites by *C. variipennis* following a blood meal in nature. It is logical to assume, however, that engorged females select locations which offer them some shelter from temperature extremes and direct solar radiation. Thus, pending further field study, average air temperatures might be reasonably used in estimating actual exposure temperatures.

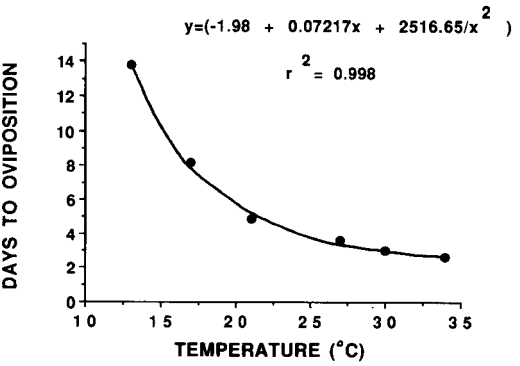

Fig. 4. Relationship between temperature and mean day of oviposition (days after blood feeding) by *Culicoides variipennis*.

The time estimates presented here for *C. variipennis* represent only the time required for egg development and oviposition after a blood meal and thus are less than the total length of the gonotrophic cycle. Time required for such things as host-seeking or location of suitable oviposition sites probably depends on the nature of the habitat, but the duration of oogenesis should constitute the bulk of the time involved in the gonotrophic cycle in most situations. This study was conducted with insects adapted to laboratory conditions. Field populations could be expected to show greater variability in days to oviposition, for instance, due to greater genetic and environmental heterogeneity.

Seasonal variability in parity rates, beyond that simply induced by voltinism pattern, has been demonstrated for certain *Culicoides* species in nature (Braverman et al. 1985, Linhares and Anderson 1989). Daily survivorship estimates derived from simple parity rates may be adjusted to reflect temperature-induced variability in the duration of the gonotrophic cycle. As a hypothetical example, a parity rate of 0.35 in spring, with an average temperature of 13°C, would generate a simple daily survivorship estimate (Davidson 1954) of approximately 0.93 ($\sqrt[13.8]{0.35}$). The same parity rate in summer, with an average temperature of 27°C, yields an estimated daily survivorship of 0.75 ($\sqrt[3.6]{0.35}$). Given the extreme sensitivity of vectorial capacity to daily survivorship, such variability should be taken into account. Studies are currently under way to determine the duration of BLU extrinsic incubation in *C. variipennis* as influenced by temperature, a necessary adjunct to field estimates of vectorial capacity of this species.

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