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Physiological Age-Grading and Survival Rate in *Culidoides* (Diptera: Ceratopogonidae) as a Means of Determining Vector Capacity

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I. Israel

- C. Abstract. The vectorial potential of Culicoides imicola, Culicoides obsoletus, and Culicoides oxystoma for bluetongue virus (BTV) in Israel in 1981-1982 was evaluated based upon their respective mean periods between blood meals, estimated survival rates between blood meals, and their expectation of infective life. Culicoides imicola was identified as the species with the highest vectorial potential because of its seasonality, presence throughout the entire year, survival rate, and expectation of infective life. The parous rate in this species peaks from mid summer to the onset of winter, a period corresponding to the bluetongue season. By the same criteria, C. obsoletus was ranked 2nd in vectorial potential. Culicoides oxystoma was ranked 3rd because of its small numbers, presence only from August to December, very limited peak survival rate, and short period of long expectation of infective life.

II. US

Changes in area of the tergal pigmentation patterns in abdominal segments 2-7 of female Culicoides variipennis were examined in relation to age after emergence, oogenesis following the 1st blood meal, oviposition, and oogenesis following the 2nd blood meal. Pigmented areas were determined in each of the 14 age groups by weighing cut-outs from enlarged photomicrographs of 10 females. By far the greatest increases in pigmentation were found to be associated with 2 periods, (1) the first 24 h after emergence and, (2) the 36 h immediately following the first blood meal. No further changes occurred after oviposition or after the 2nd blood meal. The tergal pigmentation patterns of Culicoides furens were similarly examined and analysed. Data were taken from 10 females in each of 11 age groups. There were no significant changes in the pigmented areas in any segment except 2. A significant change occurred in this segment only after the blood meal was taken and consisted of a relatively small increase in the width of the lateral and median pigment lobes. In C. furens, segment 2 is the only one in which pigment is distributed in 3 lobes.

I. Israel

The outbreak and spread of a vectored disease such as bluetongue (BT) depends on 3 major components: (1) the availability of donor(s) and susceptible host(s); (2) characteristics of the vector species, such as susceptibility to infection, capacity to transmit, population density, seasonality, geographical distribution, host preference, survival rate, and proximity of larval habitats; and (3) climatic components such as temperature, humidity, rainfall, and wind. Temperature, humidity, and rainfall influence the rate of breeding and biting of the vector; wind direction and velocity may determine whether transportation of infected vectors to remote areas will occur (Sellers 1980).

If components associated with the host, vector, and climate could be evaluated mathematically, it would be possible to forecast or at least warn of a potential BT outbreak. Some of these components have been studied in Israel since 1968. Bluetongue virus types were isolated several times in Israel exclusively from Culicoides imicola Kieffer (Braverman et al. 1981). This was expected, owing to the incrimination of this species as a vector in South Africa (DuToit 1944). The virus has been isolated also from Culicoides obsoletus Meigen in Cyprus (Mellor & Pitzolis 1979). A species of the Culicoides schultzei group was suspected by Walker (1977) to be a vector in Kenya. These 3 species are prevalent near livestock in Israel. The population density, seasonality, and geographical distribution, of the major vector (C. imicola) and other species have been studied by Braverman (1973) and Braverman & Galun (1973). Host preference studies on C. imicola and other species were also completed (Braverman et al. 1971, 1977, and unpub. data).

The present study deals with the seasonal survival rate and vectorial potential for BTV of major cattle-biting Culicoides species in Israel.

II. US

Culicoides variipennis (Coq.) is known to be a major potential vector of the virus causing Bluetongue disease of sheep and cattle in the United States (Foster et al. 1963, 1968; Jochim & Jones 1966). The pathogen causing epizootic hemorrhagic disease of deer is also known to be transmitted by this species (Jones et al. 1977; Foster et al. 1977). Culicoides furens (Poey) is a species of major economic importance as a pest in coastal areas of the Americas and Caribbean (Linley & Davies 1971) and is known to be the major vector of Mansonella ozzardi among human populations in Haiti (Ripert et al. 1977; Raccurt & Lowrie 1981).

The age structure of Culicoides populations, important in the understanding of vector competence, is amenable to study principally by 3 methods, (1) formation and persistence of follicular relics in the ovaries (Detinova 1962); (2) appearance of a burgundy red pigment in the lateral abdomen (Dyce 1969; Akey & Potter 1979), and (3) changes in the pigmented areas on each abdominal tergite (Potter & Akey 1978). The validity of these methods for distinguishing nulliparous and parous females has been evaluated for C. variipennis with both laboratory colony and field-collected specimens (Akey & Potter 1979, Potter & Akey 1978) and particularly with field populations in New York by Mullens & Schmidtman (1982). These authors were concerned with the accuracy of criteria suitable for rapid age-categorization of field collections. To date, however, quantitative methods have not been used to estimate lateral abdominal or tergal pigmentation. The changes that occur are therefore not precisely understood and there is no direct measure of their variation. In this paper we have examined and measured changes in area in the tergal pigmentation patterns of C. variipennis and C. furens in relation to age and completion of 1 and 2 gonotrophic cycles.

MATERIALS AND METHODS

I. Israel

Populations of 3 phototrophic species of Culicoides in Israel were sampled by Monks Wood suction light traps fitted with black light tubes (Philips TL 6W05) and operated from 220-V AC supplies via 12-V DC regulators. A cone made of bolting cloth of 64 mesh was attached to the bottom of each trap, ending in a screw-top plastic bottle. The bottle contained a dilute saline and detergent solution (7X, manufactured by Flow Laboratories Ltd., U.K.), which preserved the catch and simplified sorting (Walker & Boreham 1976).

Traps were placed in calf or cow sheds, about 1 m above the heads of animals and 2 m below the roof. The sheds were large, open-sided structures with slatted floors in which the animals were permanently stalled. Traps were placed near the center of the shed to minimize edge effects and the possible influence of the moon. Traps were operated at 2 locations every night, 7 nights per week, except for occasional malfunctions.

In Zimbabwe, the Culicoides spp. were sampled by a DuToit suction light trap equipped with a mercury vapor globe (HPL-N-125 WE/73/2). The trap was situated outside a netted rectangular chicken shed, very close to one of the long sides of the shed, in which chickens were kept on the ground.

Females of the dominant species at each sampling location were classified by external examination into the following categories established by Dyce (1969) (abbreviations follow Birley & Boorman, 1982): White nulliparous unfed (WNI); white nulliparous blood fed (WBF); pigmented parous unfed (RPI); pigmented parous blood fed (RBF); and semigravid plus gravid (PG). For the purpose of analysis, WNI+WBF and PRI+RBF were used and assumed to represent nulliparous and parous flies respectively, which were either actively searching for a blood-meal or had recently obtained one.

The following 2 sampling sites in Israel, described in detail by Birley et al. (1984), were chosen because of high populations of suspected vector species, and repeated isolations of BTV at these locations (Braverman et al. 1981, 1984).

1. Volcani campus, Bet-Dagan. A trap was operated on consecutive nights from 1 April 1981 to 31 March 1982 in a calf shed. The trap was activated from 3 h before sunset until 3 h after sunrise. The dominant species, C. imicola, breeds in and around animal pens in water-trough overflow, at the margins of animal sewage and drainage canals, and around puddles created by leakage from water pipes (Braverman et al. 1974, Braverman 1978). The 2nd most prevalent species, Culicoides oxystoma Kieffer (a member of the Culicoides schultzei group) breeds in sites such as drainage canals, which generally contain less organic matter than do breeding sites of C. imicola.

2. Kibbutz Kabri. A trap was operated at this location for 37 of 39 nights between 23 April to 1 June 1981. This period is believed to encompass the spring peak of C. obsoletus abundance (Braverman, unpubl. observ.). The trap was activated for 2 h. 1900-2000 h and 2000-2100 h. Sunset was at approximately 1830 h in April and 1900 h in June (Birley et al. 1984). In a 2nd study period, the trap was operated from 1800 to 0800 h during 38 of 48 nights from 16 September to 27 October 1981, a period in which the high autumn population peak of C. obsoletus is known to occur (Braverman, unpubl. observ.). The dominant species, C. obsoletus, was very numerous at times. It breeds predominantly in rotten banana stems in an adjacent large plantation owned by the kibbutz (Braverman, unpubl. observ.; Mellor & Pitzolis 1979).

A poultry farm near Mount Hampden (17°45'S, 30°55'E), Harare area (Zimbabwe) which was chosen for sampling because of the high population of C. imicola there. The farm had 75,000 chickens (Braverman & Phelps 1981). A light trap was operated there once a week at 1700 to 0800 h from 21 September 1976 to 16 September 1977.

In all trapping localities, the distance from the breeding sites of the major species to the suction light traps was assumed to be well within the flight range of these species. This was based on sampling of breeding sites and from information on other species (Lillie et al. 1981).

Method of analysis. The method has been described by Birley & Rajagopalan (1981) and Birley & Boorman (1982) and has been detailed and tested recently with some Culicoides spp. in Israel (Birley et al. 1984). According to this method: Parous biting = total biting 1 cycle previously x proportion of surviving:

$$\begin{aligned} M_t &= T_{t-u} \cdot P_u \\ T_t &= N_t + M_t \end{aligned} \quad (1)$$

where t is time in days, u is duration of the oviposition cycle in days, N_t is the number of nulliparous flies on day t , M_t is the number of parous flies on day t , T_t is the total number of nulliparous plus parous flies on day t , and P_u is the proportion of flies surviving 1 gonotrophic cycle, or the survival rate. In this method, survival rate may be estimated when the recruitment rate and population density are fluctuating rapidly. As the length of the oviposition cycle may vary between individuals, some flies may seek blood meals after 2 days, while others require 3, 4, or more days. Formula 1 can then be generalized:

$$M_t = P_2 \cdot T_{t-2} + P_3 \cdot T_{t-3} + P_4 \cdot T_{t-4} \quad (2)$$

where the proportion that have an oviposition cycle of length i days and survive i days is P_i . If the time series M_t and T_t are sampled for 25 or more consecutive nights, then an estimate of the parameters P_i may be obtained by treating equation 2 as a multiple linear regression through the origin. The mean survival rate per

oviposition cycle, P , and the mean length of the oviposition cycle, U , may then be estimated as follows:

$$\begin{aligned} P &= P_2 + P_3 + P_4 \\ U &= (2.P_2 + 3.P_3 + 4.P_4) / P \end{aligned} \quad (3)$$

When P and U have been estimated, they may be used to calculate the expectation of infective life. This is their contribution to the potential vectorial capacity of the population. If n is the extrinsic incubation period of a disease agent in the insect, which then remains infective for life, the expectation of infective life is v :

$$v = P^{n/u} / (1-P) \quad (4)$$

Vectorial capacity (Garrett-Jones 1964), V_g , defines the average number of potentially infective bites that will ultimately be delivered by all the vectors feeding upon a single host in one day:

$$V_g = (m.a.) \cdot a.p^n / -\ln p$$

where m is vector density in relation to the host; a is "host-biting habit", i.e. the probability that a vector feeds on a host in one day; P is the probability that a vector survives through one day; n is the duration of extrinsic incubation period in days; $1/(-\ln p)$ the duration of vector's life in days, after survival through the extrinsic incubation period; and T is the total host population size. Potential vector is a term used generally for a taxon that by its feeding habits and seasonality, appears capable of serving as vector. This term is not defined mathematically.

P and U values in the present study were estimated by using the SAS package available for an IBM computer. These values were calculated by treating the data of C. imicola and C. oxystoma at Bet-Dagan as running means. A length of 32 days was chosen empirically. Culicoides obsoletus from Kabri was treated in the same way, and a running mean of 20 days was chosen empirically. The expectation of infective life, v , was calculated by using an extrinsic incubation period

of 12 days (Foster et al. 1963, Lehane 1981) and the estimated values of U and P. The parous rate, P_o , from the weekly sampling data (1976/1977) for C. imicola from Zimbabwe was compared to random once-a-week sampling data on C. imicola from Bet-Dagan in 1981/1982 (which were picked from the consecutive night trappings). The parous rate is defined as the number of parous gnats divided by the number of nulliparous plus parous.

II US

Maintenance of insects and blood feeding

All females used in the study were kept at 25-27°C in 1-pint ice-cream cartons and with continuous access to 10% sucrose. The insects were bloodfed, when required, on a rabbit. Only those individuals engorged to repletion were taken for observation.

Source of material

Culicoides variipennis adults were available from a laboratory colony maintained at Vero Beach essentially by the methods of Jones et al. (1969). Culicoides furens females were collected in the field, by 2 methods. Newly emerged females were obtained from conical, galvanized steel emergence traps, from the same tidal ditch (adjacent to the laboratory) used previously by Linley et al, (1970). All females in this population are autogenous in the first gonotrophic cycle at all times of year (Linley et al. 1970). Under field conditions, most adult C. furens (73%) emerge in the morning between 0700 and 1100 h (Linley 1966a) and only 1% before 0700 h. Traps were therefore visited at about 1100 h, the collection jars (lined with moist filter paper) were changed and returned to the laboratory, and the newly emerged females (easily recognized by their appearance) removed. Fresh jars were placed on the traps only after 1600 h so that very few females would emerge

before the following morning (Linley 1966a). With this procedure, freshly collected individuals were assumed to be 0-4 h old and older groups were easily obtained except that, for these, the 4 h latitude was ignored and all the females were assumed to have emerged at 1100 h.

Since C. furnes females in the natural population at Vero Beach are all autogenous, those that attempt to bite in the field are invariably parous. Biting females taken early in the morning, when a peak in the activity of this species occurs (Kettle 1969), almost always have large, saccate dilatations in the ovaries, indicating very recent oviposition (Linley 1965). These females were collected with an aspirator in the early morning. They provided a convenient source of material for the groups to be studied after oviposition and also for groups after the blood meal, since they readily took blood when later placed on a human host. From ambient field temperatures and the known duration of the gonotrophic cycle (Linley 1966b), it was assumed that the parous females were 3 days old and, since their ovaries contained very large dilatations, that this was their age at oviposition. Blood was given 24 h later, at an assumed age of 4 days.

Age groups studied

For C. variipennis there were 14 age groups and for C. furens there were 11. The ages, treatments and temporal relationships of the groups are clarified for both species in Fig. 1. Letter codes are assigned to facilitate reference in the tables and text. In the case of C. variipennis, a latitude of 12 h was unavoidable in the case of the 2 groups kept after oviposition, because the exact time of laying could not be determined for each female.

Measurement of pigmentation

To observe and measure area changes in the tergal pigmentation patterns, permanent preparations were made of 10 females representative of each age group for each species. Insects were fixed in Carnoy for at least 2 h and were then removed to a small (3-4 mm square) piece of wet filter paper on a slide. The abdomen was cut off as closely as possible to the thorax and the latter discarded. Each abdomen was then sliced cleanly with a fragment of razor blade to separate dorsal and ventral halves. These were cleaned of all the internal tissue, including abdominal epithelium, then dehydrated and mounted in Permount with the external cuticular surface uppermost. A minimum of mountant was used to promote optimum flattening. It was necessary to remove all internal tissue to obtain clear photographs showing the pigment boundaries. To estimate the tergal pigmented areas, enlargements at constant magnification were made from photomicrographs and the pigmented areas cut out and weighed. All work was done by one individual to minimize inconsistencies in the interpretation of pigment area boundaries. As a check for consistency of the photographic paper, a rectangle of fixed dimensions was cut from each of 3 sheets in each box of paper opened and the weights compared. Negligible differences were found. For each female, the tergal pigment areas on segments 2-7, inclusive, were evaluated.

Other measurements

In addition to determinations of pigmented area, certain linear measurements were made of the pigmentation patterns. For C. variipennis, measurements were made in segments 2-5, inclusive, for the 4 groups earliest to emerge (see Fig. 9). During this 48 h period, the pigment patterns appear as 3 "lobes", 1 median and 2 lateral, projecting posteriorly from the anterior edge (Fig. 10). The width of each lobe and the total distance across the anterior edge was measured

at the widest points (Fig. 10). Later than 48 h after emergence, the lobes begin to coalesce in many insects and further consideration of these measurements was not considered useful. Measurements of the lobes were also made in C. furens, as described later.

RESULTS

I. Israel

The seasonality curve of C. imicola (Fig. 1) shows a build-up from the 2nd half of July. A fluctuating but high population level was maintained till December, with peak between the 2nd half of August and the 1st half of September. Culicoides oxystoma (Fig. 2) had a limited seasonality between July and December, with a high population level maintained from the 2nd half of August till the end of November. The estimate of the mean period, in days, between blood meals of C. imicola at Bet-Dagan was longest in April, July, September, and December (ranging from 4.4 to 4.7 days) and shortest in June, August, January, and February (about 3 days) (Fig. 3). Culicoides oxystoma had a short season from August to December (Fig. 2). In this period it generally followed the pattern of C. imicola (Fig. 3). The 2 peak levels ranged from 4.8 to 5 days, whereas the low levels were around 3.6 days.

The estimated survival rates between blood meals of C. imicola and C. oxystoma at Bet-Dagan are shown in Fig. 4. Culicoides imicola showed high values of around 0.4 between April to October, with 2 high peaks of 0.75 in August. Except for January and February, the survival rate values in the rest of the year were rather high and ranged from 0.2 to 0.4. Culicoides oxystoma showed a peak of 0.9 for a short period at the end of August, and then declined and terminated in December at a value of 0.2. The estimated mean periods between blood meals in C. obsoletus at Kabri are shown in Fig. 5. The spring graph shows a mild rise to

a value of 4.0, whereas the autumn graph shows a distinct pattern of values ranging around 4.5 from 25 September until 7 October, and then a shift to a nearly constant value of 3.0. The estimated survival rate between blood meals for C. obsoletus (Fig. 6) is generally higher in the spring than in the autumn, except for a short peak period in mid-October with a value of 0.45. The expectation of infective life (Table 1) of C. imicola at Bet-Dagan peaked to its highest value of about 1.8 in August, whereas an extremely high value of 7.3 was obtained for C. oxystoma during a short period in September. Culicoides obsoletus in Kabri showed high values for expectation of infective life of about 1 to 1.3 for a very short time in October. The parous rate curve for C. imicola from Mt. Hampden, Zimbabwe (Fig. 7), showed 2 peaks, 1 in October-November and the 2nd, slightly higher but shorter, in March. The comparable curve for parous rate of C. imicola from Bet-Dagan showed, except for a brief decline in July, a general increase from April to October and then a decline (Fig. 8).

II. U.S.

Tergal pigment areas in C. variipennis

The mean weights proportional to the tergal pigment areas for each age group are shown in Table 1. Figure 11 allows the changes occurring in each segment to be seen and compared easily. A representative female abdomen from each age group is shown in Figs. 12 and 13.

The changes in tergal pigmented area in C. variipennis occurred in two stages. The first was associated with the first 24 h of life (ae), the second with the first blood meal (abl) and subsequent egg development. In females less than 4 h old (Fig. 12), the pigment was generally fainter than in older flies and may be almost invisible or completely absent, particularly in segment 6 and 7. Of the 10 females examined in this group, 4 had only traces of pigment visible in these 2 segments, almost all of it associated with the median lobe.

In the period represented by the first 3 groups, up to 24 h after emergence, the pigmented area increased in all segments (Fig. 11) with the greatest increases in segments 2, 6 and 7. By analysis of variance, these changes were highly significant for all segments except 4, which did not quite attain significance at the 5 percent level. Although the data (Fig. 11) suggested some continued, progressive increase in pigmented area in all segments from 24- 120 h after emergence, these changes were not found to be significant in any segment.

The typical appearance (Fig. 12) was for the lobes in segments 2, 3, 4 and 5 to be visually distinct, even though they were occasionally joined by narrow bridges of pigment posteriorly (more commonly in segments 4 and 5). In most flies, however, the pigment area in segment 6 was partially or wholly filled in and in segment 7 it was almost invariably so. Occasional exceptions to this general pattern will be discussed later. Females kept without blood were not examined beyond 120 h after emergence.

Changes in the pigmented areas took place within 24 h after a blood meal and were virtually complete by 48 h, at which time the eggs reached maturity (at the experimental temperature). By 24 h after the blood meal, pigment began to fill in between the median and lateral lobes, so that only narrow unpigmented strips sometimes persisted, especially in segment 3, 4 and 5 (Fig. 13). Even these were usually occluded by 48 h after the blood meal.

At this time, pigment in all segments appeared as a solid rectangular patch (Fig. 13). An increase in pigmented area was measured in all segments after the 1st blood meal (Fig. 11), but was least in segment 7 and by far the greatest in segment 2. Beyond 48 h after blood there was little further change and none even when a second blood meal was given (ab2) and the females examined 48 h later (Fig. 11, Fig. 13). When the data groups (excluding 24 ab1) were

examined by analysis of variance, no significant changes in pigmented areas were found in any segment.

Measurement of lobes anterior margin in *Culicoides variipennis*.

In very young *C. variipennis* females (Fig 12, 0-4 ae, 12 ae) the lateral lobes, particularly in segments 3, 4 and 5, appeared to spread posteriorly relative to their points of origin on the anterior margin. This appearance, unfortunately not exclusively characteristic of very young individuals, was observed because the lateral lobes were somewhat tapered anteriorly along their lateral margins (Fig. 12) and also because the anterior margin was narrowest at this time. When changes in length of the anterior margin were plotted (Fig. 14), the greatest increase was seen to occur in the first 24 h after emergence, with further slight progressive increase after this time. Statistically, the overall changes were significant by analysis of variance for segments 2, 3 and 4 (but not 5), but none was significant when only the data for 24 ae and later were included.

The 2 lateral lobes were considered equivalent and the data were combined to derive the mean widths, shown for the first 48 h after emergence in Fig. 15. By far the greatest increase in width occurred in the first 12 h after emergence in all segments measured (2, 3, 4, 5). The differences in lobe width from 0-4 ae to 12 ae seemed progressively greater from segment 2 to 5 (Fig. 15). These changes were all highly significant, but excluding data for 0-4 ae, only segment 3 showed significant differences between the 3 older groups, presumably because of the rather low value for mean width obtained in the 24 ae group. In segment 5, the change in width from 0-4 ae to 12 ae and later was so great as to provide a basis for separation of very young females

from those 12 h and older with 95% certainty. The mean ($\pm$ SD) for 0-4 ae was 35.09 ± 8.11 and for the older 3 groups combined was 70.67 ± 9.67 . There was, consequently, no overlap at $1.96 \times$ SD to either side of the respective means. Results for the median lobe were generally similar, with highly significant increases in width occurring in the 4 segments measured up to 48 ae. Major change again took place in the first 12 h after emergence, but with segments 2 and 5 showing continued increase from 12 ae to 48 ae.

Pattern types in *Culicoides variipennis*

In their original description of the tergal pigmentation changes in *C. variipennis*, Potter & Akey (1978) classified the patterns of abdominal tergites 2 and 3 into 6 types. Table 3 shows the results of applying similar criteria to the specimens examined here. The laboratory colony at Vero Beach is the same as the Texas strain referred to by Potter & Akey (1978). In the 7 age groups (comprising 70 females) up to 5 days after emergence but before blood (Table 2), 58 of the females (83%) were judged Type I (nulliparous); 12 (17%) could not be assigned certainly as nulliparous. Potter & Akey judged only 2% (9/491) of their sample (females up to 25 days old) as doubtfully nulliparous and, surprisingly, all 9 of these individuals were Type V, with considerably more pigment than the mostly "intermediate" individuals seen here (Table 2). When a blood meal was given, Potter & Akey found most females showing Type IV and V patterns, even though only 6 h had elapsed since the blood meal. Overall, a proportion of 85% (447/517) was in Types IV, V and VI and therefore judged as parous. This result is the same as obtained here (Table 2), with 60/ 70 (86%) classified as parous and 14% therefore remaining uncertain. Among the blood-fed females evaluated here, 2 remained as Type I (Table 2), while for comparable times after the blood meal, Potter & Akey

(1978) found only 1 in a much larger sample.

Tergal pigment areas in *Culicoides furens*

The tergal pigmentation patterns of 2 *C. furens* females, age 48 ae, are shown as examples in Fig. 16. In this species, the pigment was considerably lighter than in *C. variipennis*. Also, with the exception of segment 2, the pigment was not at any time distributed in 3 distinct lobes (Potter & Akey, 1978) but was found in narrowly rectangular patches (especially posteriorly), which often had rounded posterior lateral margins (Fig. 16). The trilobed appearance in segment 2 was quite different from the other segments and was similar in appearance to *C. variipennis*, especially segments 2-4 of this species, prior to a blood meal.

The mean weights proportional to the pigmented areas in each segment for each *C. furens* age group are shown in Fig. 17. Analysis of variance showed that there were no significant differences in areas pigmented between age groups in any segment except 2. In this segment, when data from all 11 groups were included, a highly significant F-ratio was found ($F = 3.004$, d.f. = 10,99 $P < 0.005$), but not when the 2 groups after the blood meal (24 abl, 72 abl) were excluded ($F = 1.191$, d.f. = 8,81, $P > 0.300$). The only change in pigmented area that took place, therefore, was in segment 2, and it occurred only after the first blood meal (Fig. 17). No measureable change occurred in any segment during the first 72 h when the first batch of eggs was being matured autogenously. Even though the data showed that pigment in segment 2 increased in area to some extent when blood was taken, it was not easy to detect this effect visually. The pigment area did not fill in completely to form a rectangular patch (as *C. variipennis*) and only a slight widening of the 3 lobes seemed apparent in most of the mounted specimens. To test for this

effect, the diameters of the lateral and median lobes (at their widest points, see Fig. 10) were measured with an ocular micrometer for groups 72 ae, 96 ae and 120 ae (representative of the condition before a blood meal) and for groups 24 abl and 72 abl (representative of the condition after a blood meal). The mean widths and results of statistical comparisons are shown in Table 3 (the lateral lobes were considered equivalent and the data combined for analysis). A highly significant increase took place in the mean width in both lateral and median lobes. There was therefore some increase in pigmented area in segment 2 when the 1st blood meal was taken and digested, but the change was not easily seen because the pigment was quite pale, its boundaries poorly defined and, with occasional exceptions (see discussion), the pigment area did not completely fill in to produce a clearly rectangular or trapezoid patch.

DISCUSSION

I. Israel

As the purpose of this study was to determine and rank the survival rate and the expectation of infective life of suspected vector species, it is not of major importance that a biased sampling method, i. e., suction light traps, was used. Light traps have the advantage of collecting a wide range of species (Acuff 1976) and large numbers of Culicoides spp. Unbiased methods like truck suction traps, often catch too few insects to allow determination of survival rate according to the Birley & Rajagopalan (1981) method.

There are differing reports on the extrinsic incubation period of BTV in the various vectors. DuToit (1944), working with C. imicola in South Africa, assumed an incubation period of 10 days, while E.M. Nevill (pers. commun.), experimenting with C. imicola and other species in South Africa, assumed an

incubation period of 7 to 11 days. Lehane (1981) reported from Australia that for C. brevitarsis Kieffer (which is closely related to Culicoides imicola) and Culicoides No. 5 (these 3 belong to the same subgenus, Avaritia), an incubation period of 10 to 12 days seemed appropriate. Foster et al. (1963), Barber et al. (1980), and R.H. Jones (pers. commun.) reported that C. variipennis can transmit BTV after an incubation period of 7 to 10 days, while Luedke et al. (1976) noted an incubation period of 15 to 21 days for the same species. As the temperature during the incubation period was not always noted by these authors, it is impossible to compare these periods in terms of day-degrees or to decide if the variation is due to temperature. One may assume that various vector species may have different incubation periods, and that laboratory reared C. variipennis might differ from their wild counterparts. Our results show that in October, which is generally the peak month of the BT season in Israel, the mean period between blood meals of C. imicola was Ca. 4.2 days. Mirzaeva (1974) and Skierska (1974) noted that some Culicoides spp. can have 3 to 4 gonotrophic cycles. With this in mind and considering the above-mentioned information, it was decided that for calculation of the expectation of infective life the appropriate incubation period (n) for BTV would be 12 days. The seasonality curve for C. imicola (Fig. 1) generally shows a fluctuating population build-up from the end of July till the end of November. This pattern differs from previous observations (Braverman & Galun 1973), which clearly show a small peak in the spring and a 2nd large one in September-October. It is difficult to compare seasonality curves of different years because seasons do not start each year on the same date; however, it is reasonable

to assume that the present data are more reliable, since they are based on consecutive nightly catches from a site rich in C. imicola, while previous observations (Braverman & Galun 1973) were based on 1 to 3 nights of trapping per week at Bet-Dagan at a site less rich in C. imicola.

Figure 2 presents the first information on the seasonality of C. oxystoma in Israel. This species has a short season, from August to December, and its virtual absence during the rest of the year excludes it as an overwintering host for BTV. From the standpoint of seasonality and expectation of infective life, the optimal season in Israel for the vector C. imicola appears to be August-September (Fig. 1, Table 1). During this period the average time between blood meals was from 3.3 and 4.4 days (Fig. 3). The BT season in Israel is from mid-summer (July) till onset of winter half way through November, with most cases occurring from September to November (Braverman & Galun 1973). According to the seasonality, survival rate, and expectation of infective life data for C. imicola presented here (Fig. 1 & 4, Table 1), the peak month for BT in 1981 would be assumed to be September, i.e., about 2 weeks after these peaks in August. Earlier data (Braverman & Galun 1973) show that BT disease can occur in every month of the year. This is understandable because it takes only the bite of an individual Culicoides to transmit the disease (Luedke et al. 1976), but the chances that enough infected individuals will survive beyond the main BT season (July to November) are small, especially if other factors are considered (e.g., infection and transmission rates of the vector in the field, number of donors, climatic conditions). Nonetheless, as transovarial transmission is a remote possibility (Jones & Foster 1971), the chances for C. imicola to act as an overwintering agent for BTV in Israel are greater than in the Onderstepoort area of South Africa (28°11'E, 25°50'S) (Nevill 1971) because midges of this species are frequently caught in suction light traps

during the winter in Israel but not in South Africa. Since factors affecting survival differ from year to year, the autumn peak in Fig. 3 may in other years be earlier in November and, if favorable conditions prevail, the spring peak may start earlier in March. Figure 4 shows the estimated survival rate between blood meals of C. imicola to be at its highest at the end of August. This very high value (around 0.75) corresponded to the long expectation of infective life at the end of August (Table 1) and may be associated with the peak of BT 2 weeks later in September. The decline in survival rate in mid-August cannot be explained by prevailing local climatic conditions, but rather by termination of a generation, before the mass emergence of a new one. The mean period between blood meals of C. oxystoma at its appearance between August to December showed a pattern similar to that of C. imicola (Fig. 3). The single peak in survival rate of C. oxystoma (Fig. 4) was higher (0.9) and later than that of C. imicola and was within the peak of the BT season. The expectation of infective life of C. oxystoma in September (Table 1) was 7.31, the highest of the 3 species. Because this factor contributes exponentially to expected infective life, and because C. oxystoma peaked from August to October, this species could serve as an important vector during that period. However, its short season and distribution indicate that C. oxystoma should be ranked 3rd as a potential vector. Figure 5 shows that for C. obsoletus at Kabri there was a slightly higher value in the mean period between blood meals in autumn as compared to the value in the spring. The survival rate values (Fig. 6) of C. obsoletus in the autumn showed a high value of 0.45 only for a short time in mid-October, as compared to the longer spring peak of about 0.27. The high peak of the autumn was well within the BT season, but somewhat later than the peak in the expectation of infective life (Table 1), which is in the beginning of October. The expectation of infective life data suggest that the capability of C. obsoletus

to transmit BT at Kabri in mid-October is 61 times greater than at the end of May. Because of the presence of C. obsoletus throughout the entire year (Bravenman et al. 1976), this species should be considered 2nd in importance as a potential vector and as an overwintering agent. In the more temperate and less stable weather conditions of England, where the number of gnats on the wing may be subject to more dramatic peaks in population changes, C. obsoletus was considered a potentially significant vector only during the short period of July and August (Birley & Boorman 1982), 2 to 3 months earlier than in Israel. This is understandable owing to the cooler conditions in England.

The parous rate of C. imicola in Zimbabwe (Fig. 7) showed a bimodal pattern, while in Israel (Fig. 8) the spring peak was less conspicuous. The 2 peaks in Zimbabwe were before, and toward the end of the rainy season. The peak before the rainy season reflects a lower rate of recruitment from the shrinking breeding sites, and the resulting enhancement of the parous portion of the C. imicola population. The peak at the end of the rainy season is also a result of higher numbers of parous insects, as the cool weather inhibits new recruitment of nullipars. According to Nevill (1971) and DuToit (1962), the main BT season in South Africa and in Zimbabwe (R.J. Phelps, pers. commun.), is mid-February to May. At this time the numbers of C. imicola were still substantial, as was the parous rate (Fig. 7).

It seems reasonable to consider the patterns of high parous rate, survival rate, and vectorial capacity during autumn in Israel, and the post-rainy-season peak in parous rate in Zimbabwe, as generally valid. However in other years and other regions, the factors affecting the vector(s) and donor(s) (such as weather conditions) might differ, with a resulting temporary shift in vectorial capacity. To establish a reliable warning system against BT disease, therefore, permanent

trapping stations should be located at the major livestock areas, taking into account the prevailing winds, which might carry infected vectors. Trapping stations should operate throughout the vector season. By doing this, survival rate and vectorial capacity of the potential vector(s) could be monitored along with types of BTV isolated from the insects. By obtaining advance information on the viral serotype, it would be possible to use the appropriate serotype vaccine (DuToit 1962) to control oncoming BT disease. Advance information indicating high vectorial capacity would permit protection of sensitive animals from the vector, either by spraying with repellents or by spraying the breeding sites with insecticide. By using reliable survival rate data, it should be possible to divide the country into areas of varying degrees of risk for Culicoides vectored diseases. Specific regions could thus be designated as preferable for breeding animals.

II. U.S.

Our main purpose in the study of C. variipennis was to provide a quantitative and reasonably complete description of the tergal pigment patterns and their relationship to age and oogenesis. There was, however, a possibility of deriving quantitative criteria that would allow individual females to be assigned to narrower ranges of age, thus providing greater resolution of population age structure. This has not proven possible (and to know this is itself valuable), mainly for 2 reasons. First, the pigment patterns and areas are quite variable within a particular age and parity condition. In a few females the pigment patterns change scarcely at all from the nulliparous condition, even after the blood meal is fully digested and the eggs mature (Table 2). Second, changes that do occur are confined within relatively narrow boundaries of time (Fig. 3), principally within the first 24 h after emergence and within 36 h of the 1st blood meal. We encountered, also, some difficulty in deciding the boundaries of the pigmented areas. Different persons

tend to have different perceptions of where the edges of the pigmented areas should be drawn. This applies less to flies showing the parous condition (Fig. 13), where the rectangular dark patches are quite distinct, but in nulliparous females the edges of the lobes may often be ragged and less clearly delineated (Fig. 12). Pigment in these areas (visible under enlargement in Fig. 10) tends to appear progressively as a series of small patches, which may be entirely or partially separated from the main pigment area. A further complication is that pigment is not uniformly dark, as seen particularly in segments 4, 5 and 6 of some specimens taken before a blood meal (Fig. 12). Comparing Figs. 12 and 13, the pigment generally seems darker after the blood meal. Individual interpretations of the pigment patterns are bound to be affected by these factors, which will influence the assignment of patterns to particular types. The scoring of Potter & Akey types in Table 2, for example, was done by J.R.L., but the specimens were also typed by the technician who prepared them and, with the exception of the youngest insects (0-4 ae), she consistently scored some flies in every group at a higher type score.

Despite these problems, which should not be overemphasized, abdominal tergite patterns are a useful confirmatory index of parity in C. variipennis. In shrivelled specimens, it is frequently easier to see the tergite pattern than to decide whether any lateral abdominal pigment (Dyce 1969) is present. It is important, however, to understand variations in the tergal pigmentation peculiar to the population one wishes to study. This is well illustrated by Mullens & Schmidtman (1982), who looked at C. variipennis populations in New York and found 65% and 23%, respectively, of nulliparous females with the Type II and III intermediate (during oogenesis) conditions. These results differ substantially from those reported by Potter & Akey (1978) with the Texas strain.

As is evident from the results, the tergal pigmentation of C. furens females is rather different in form and considerably less liable to change with age and experience of oogenesis than in C. variipennis. Three distinct lobes of pigment are seen before oogenesis in C. variipennis, particularly in segments 2-6. Culicoides furens, in contrast, has lobes only in segment 2 and no significant change in pigmented area occurred in any other segment (3-7) as the flies matured eggs autogenously, were kept 4-5 days after oviposition, or matured a second batch of eggs following their first blood meal. The lateral and median lobes in segment 2 increase in width after blood is taken (Table 4). This change, however, is not obvious to the eye and the lobe widths are in any case difficult to assess objectively because of the paleness of the pigment and its indistinct boundaries, especially along the median margins of the lateral lobes. There are also occasional individuals in which pigment in segment 2 is almost completely filled in before blood is taken. Three of these were found in the unfed groups used in Table 4 and account for the taking of only 54 and 27 lateral and median measurements, respectively. Overall, our results show that tergal pigmentation is of no value for determination of age or parity in C. furens.

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g. Publications

Linley, J.R. and Y. Braverman. (In press). The tergal pigmentation patterns of Culicoides variipennis and Culicoides furens (Diptera: Ceratopogonidae). J. Med. Ent.

Linley, J.R. and Y. Braverman. (In preparation). The lateral abdominal pigmentation of Culicoides variipennis and Culicoides furens (Diptera: Ceratopogonidae).

Braverman, Y., Linley, J.R., Marcus, R. and Frish, K. (In press). Seasonal survival and expectation of infective life of Culicoides spp. (Diptera: Ceratopogonidae) in Israel with implications for bluetongue, virus transmission, and a comparison of the parous rate in C. imicola from Israel and Zimbabwe. J. Med. Ent.

Braverman, Y., Linley, J.R. and Marcus, R. (In preparation) Voltinism of adult Culicoides imicola and three other Culicoides spp., taking account of the lunar phase.

e. Cooperation

The known methods of age grading were screened for their adaptation to Culicoides spp., a procedure which was done mainly in the U.S. laboratory. The most promising method was selected for implementation in the field in Israel for determining survival rate of the major vector species. From these values the expected infective life of the tested vector species for bluetongue disease was calculated.

f. Benefit to agriculture

It was determined quantitatively that among the three major mammal feeding Culicoides spp. associated with livestock, C. imicola is the most efficient vector of bluetongue, due to its seasonality and greater survival. On the basis of the information gained, reliable criteria were provided for dividing the country into safe and hazardous zones regarding bluetongue and Akabane. Safe zones could be designated for breeding cattle for export. From the accurate seasonality and survival rate pattern, as determined for the three major Culicoides spp. associated with livestock, the time for vaccination can be adapted accordingly.

Table 1. Range of the expectation of infective life in C. imicola and C. oxystoma at Bet-Dagan, and in C. obsoletus at Kabri, Israel.

	Dates	Expectation of infective life	
		Range	Mean
Bet-Dagan			
<u>C. imicola</u>	16 Apr. - 30 Jul. 81	0.000035-0.75	0.105
<u>C. imicola</u>	4 Aug. - 24 Aug. 81	0.034 - 1.786	0.725
<u>C. imicola</u>	29 Aug. 81 - 17 Mar. 82	0.000042 - 0.117	0.027
<u>C. oxystoma</u>	29 Aug. - 18 Sep. 81	0.22 - 7.31	2.036
<u>C. oxystoma</u>	23 Sep. - 27 Dec. 81	0.0022 - 0.11	0.03
Kabri			
<u>C. obsoletus</u>	2 May - 22 May 81	0.0001648 - 0.021696	0.037
	25 Sep. - 2 Oct. 81	0.002889 - 0.00591	0.005
	3 Oct. - 6 Oct. 81	0.9868 - 1.343	1.254
	7 Oct. - 17 Oct. 81	0.000335 - 0.97119	0.017

Table 2. Culicoides variipennis, weights proportional to the tergal pigmented areas for each group.

Area in $\text{mm}^2 = \text{wt.} \times 0.1384$. $n = 10$ in all cases. ae, after emergence; ab1, after 1st blood meal;

aol, after 1st oviposition; ab2, after 2nd blood meal.

Age group	Weight (gm) of pigmented area ($\pm$ SE)						
	Segment						
	2	3	4	5	6	7	
0-4	ae	0.140 $\pm$ 0.011	0.176 $\pm$ 0.012	0.170 $\pm$ 0.011	0.149 $\pm$ 0.008	0.087 $\pm$ 0.016	0.046 $\pm$ 0.015
12	ae	0.183 $\pm$ 0.010	0.183 $\pm$ 0.008	0.188 $\pm$ 0.009	0.174 $\pm$ 0.006	0.127 $\pm$ 0.010	0.113 $\pm$ 0.018
24	ae	0.205 $\pm$ 0.012	0.214 $\pm$ 0.008	0.206 $\pm$ 0.009	0.195 $\pm$ 0.008	0.165 $\pm$ 0.012	0.166 $\pm$ 0.016
48	ae	0.193 $\pm$ 0.013	0.203 $\pm$ 0.013	0.198 $\pm$ 0.014	0.185 $\pm$ 0.011	0.180 $\pm$ 0.012	0.179 $\pm$ 0.009
72	ae	0.208 $\pm$ 0.014	0.236 $\pm$ 0.012	0.209 $\pm$ 0.011	0.210 $\pm$ 0.014	0.183 $\pm$ 0.012	0.179 $\pm$ 0.013
96	ae	0.207 $\pm$ 0.012	0.210 $\pm$ 0.010	0.202 $\pm$ 0.012	0.194 $\pm$ 0.008	0.189 $\pm$ 0.009	0.190 $\pm$ 0.008
120	ae	0.221 $\pm$ 0.019	0.223 $\pm$ 0.011	0.212 $\pm$ 0.010	0.203 $\pm$ 0.010	0.185 $\pm$ 0.006	0.193 $\pm$ 0.008
24	ab1	0.268 $\pm$ 0.014	0.250 $\pm$ 0.010	0.243 $\pm$ 0.009	0.237 $\pm$ 0.011	0.220 $\pm$ 0.011	0.215 $\pm$ 0.010
48	ab1	0.318 $\pm$ 0.013	0.262 $\pm$ 0.011	0.248 $\pm$ 0.014	0.242 $\pm$ 0.014	0.223 $\pm$ 0.011	0.204 $\pm$ 0.011
72	ab1	0.335 $\pm$ 0.006	0.273 $\pm$ 0.008	0.251 $\pm$ 0.008	0.237 $\pm$ 0.008	0.215 $\pm$ 0.007	0.187 $\pm$ 0.009
96	ab1	0.358 $\pm$ 0.014	0.286 $\pm$ 0.014	0.265 $\pm$ 0.015	0.261 $\pm$ 0.013	0.249 $\pm$ 0.018	0.216 $\pm$ 0.007
24-36	aol	0.324 $\pm$ 0.024	0.256 $\pm$ 0.021	0.238 $\pm$ 0.021	0.233 $\pm$ 0.020	0.214 $\pm$ 0.016	0.207 $\pm$ 0.014
96-108	aol	0.350 $\pm$ 0.023	0.271 $\pm$ 0.015	0.251 $\pm$ 0.015	0.244 $\pm$ 0.013	0.228 $\pm$ 0.009	0.212 $\pm$ 0.010
48	ab2	0.319 $\pm$ 0.015	0.258 $\pm$ 0.009	0.248 $\pm$ 0.011	0.237 $\pm$ 0.009	0.227 $\pm$ 0.010	0.200 $\pm$ 0.010

Table 3. The specimens of the different age groups classified according to types defined by Potter and Akey (1978)

Age group	Numbers of type					
	I	II	III	IV	V	VI
	nulliparous	intermediate	during oogenesis	parous		
0-4 ae	10					
12 ae	9	1				
24 ae	9	1				
48 ae	8	2				
72 ae	7	2		1		
96 ae	7	1	2			
120 ae	8 (58)	1 (8)	1 (3)	(1)*		
24 abl	1	1	1	5		2
48 abl				3	2	5
72 abl	1			4	1	4
96 abl		1			5	4
24-36 aol		1		2		7
96-108 aol		1	1		2	6
48 ab2	(2)	1 (5)	1 (3)	(14)	4 (14)	4 (32)†

* Totals, 0-4 ae to 120 ae inclusive

† Totals, 24 abl to 48 ab2 inclusive

Table 4. Culicoides furens, comparison of the width of the lateral and median lobes of badominal segment 2, before and after the blood meal

Mean Width ($\pm$ SE) *						
(m)						
		Before blood		After blood		
Lobes	n	(Groups 72,96,120 ae)	n	(Groups 24,72 abl)	Difference	t
						p
Lateral	54	28.95 $\pm$ 0.69	40	30.01 $\pm$ 1.47	7.06	4.665 < 0.001
Median	27	30.59 $\pm$ 0.94	20	34.09 $\pm$ 1.42	3.50	2.134 < 0.02

* at widest point

FIGURE LEGENDS

- Fig. 1 Numbers of nulliparous + parous C. imicola sampled at Bet-Dagan. Solid line is 15-day running mean.
- Fig. 2 Numbers of nulliparous + parous C. oxystoma sampled at Bet-Dagan. Solid line is 15-day running mean.
- Fig. 3 Estimate of the mean period between blood meals of C. imicola (●) and C. oxystoma (▲), caught daily in a calf shed at Bet-Dagan in 1981-1982. 32-day running mean.
- Fig. 4 Estimate of the survival rate between blood meals of C. imicola (●) and C. oxystoma (▲), caught daily in calf shed at Bet-Dagan in 1981-1982. 32-day running mean.
- Fig. 5 Estimate of the mean period between blood meals of C. obsoletus caught daily in a calf shed at Kabri in the spring (23 April to 1 June 1981) and in the autumn (16 September to 27 October 1981). 20-day running mean.
- Fig. 6 Estimate of the survival rate between blood meals of C. obsoletus caught daily in a calf shed at Kabri in the spring (23 April to 1 June 1981) and in the autumn (16 September to 27 October 1981). 20-day running mean.
- Fig. 7 Average monthly parous rate of C. imicola caught once a week at a chicken house in Mt Hampden, Zimbabwe in 1976-1977.
- Fig. 8 Average monthly parous rate of C. imicola caught in calf shed at Bet-Dagan in 1981-1982, based on once-a-week sampling.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5

Fig. 6.

Fig. 7.

Fig. 8.

C. variipennis

After emergence (ae) (h)	After blood meal (ab1) (h)	After 1st oviposition (aol) (h)	After 2nd blood meal (ab2) (h)
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4			
12			
24			
48	1st blood meal		
72	24		
96	48		
120	72	oviposition	
	96	24-36	2nd blood meal
			48
		96-108	

C. furens

After emergence (ae) (h)	After 1st oviposition (aol) (h)	After 1st blood meal (ab1) (h)
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0-4		
12		
24		
48		
72	oviposition (autogeny)	
96	24-36	1st blood meal
120		24
	96-108	72

Fig. 9. Treatments and time relationships of the C. variipennis and C. furens age groups selected for observation.

Fig. 10. Enlarged segment 3 pigment pattern of a fly 48 h after emergence to define anterior margin (am), lateral lobe (ll) and median lobe (ml).

Fig. 11. Mean weights (and 95% confidence intervals) proportional to tergal pigmented areas, segments 2-7, for all age groups. ●—●, after emergence, but before blood meal; ○---○, after the first blood meal (b_1) and oviposition (o). Point not connected to dashed line is at 48 h after second blood meal (b_2).

Fig. 12. Representative complete tergal pigmentation patterns after emergence but before a blood meal. Codes identified in Table 1.

Fig. 13. Representative complete tergal pigmentation patterns after the first blood meal, after oviposition, and after the second blood meal. Codes defined in Table 1.

Fig. 14. Mean lengths (and 95% confidence intervals) of the anterior margins of the pigmented areas, segments 2-5, for all age groups. Conventions as in Fig. 2.

Fig. 15. Mean widths (and 95% confidence intervals) of the lateral lobes (ll) and median lobes (ml), segments 2-5, from 0-4 to 48 h after emergence.

Fig. 16. Tergal pigmentation in 2 of the *C. furens* females at 48ae to show form and variation of the pigment areas.

Fig. 17. Mean weights (and 95% confidence intervals) proportional to tergal pigmented areas, segments 2-7, for all age groups. Solid symbols - after emergence (during autogenous maturation of eggs); dashed symbols - after oviposition (o); dotted symbols - after the first blood meal (b₁).