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Short communication

Life-history parameters of *Culicoides* (*Avaritia*) *imicola* Kieffer in the laboratory at different rearing temperatures

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ABSTRACT

This laboratory study investigates the sub-adult developmental cycle of field collected *Culicoides* (*Avaritia*) *imicola* Kieffer (Diptera; Ceratopogonidae). The period required from blood-feeding field-collected females to the production of progeny adults occupied 34–56 days at 20 °C, 15–21 days at 25 °C and 11–16 days at 28 °C, demonstrating clear temperature dependence. When reared at 28 °C, *C. imicola* demonstrated higher variability in fecundity (between 2.4 and 20.6 eggs/female) and lower hatching rates (50.0–62.2%), although larval survival rates to pupation were low at all temperatures (20–30%). Similarly, the mean emergence rate from pupae was the highest at lower temperatures. These results highlight the difficulty in establishing and maintaining a laboratory colony of this species from field-collected material and results are discussed in reference to future research directions that may aid this process.

1. Introduction

Culicoides (*Avaritia*) *imicola* Kieffer (Diptera: Ceratopogonidae) has been implicated in the transmission of several arboviral pathogens of international importance, including bluetongue virus and African horse sickness virus (reviewed in Mellor et al., 2000). This species is one of the most widely distributed *Culicoides* species in the world; its distribution extends broadly across the Afrotropical/Indomalayan ecozones, from the southern-most tip of Africa northwards to 44°N (Mellor, 2004), into southern Europe and thence eastwards to Laos, Vietnam and southern China (Meiswinkel, 1989; Meiswinkel et al., 2004). Due to its host preference of large mammals, close association with farmed ruminants and Equids (Meiswinkel et al., 2004) and demonstrated ability to transmit BTV (Du Toit, 1944), *C. imicola* is hence commonly regarded as the primary vector of *Culicoides*-borne livestock pathogens in much of its distribution.

Despite this importance, little is known regarding the life-history parameters of this species. To date, field and laboratory-based experimentation has been hampered both by the small size (average adult wing length <1 mm) and by the vulnerability of all life stages to physical damage during collection. These difficulties are exacerbated by a lack of accurate age grading techniques, beyond the division into parous and nulliparous adult female individuals, made upon abdominal pigmentation (Dyce, 1969). Laboratory colonisation would allow some of these issues to be addressed by providing individuals of known physiological status and age for use in a wide range of vector competence and vector capacity studies.

While only a very few *Culicoides* species have been successfully cultured in the laboratory (reviewed in Hunt, 1994; Carpenter, 2001), those that have, e.g. *Culicoides* (*Monoculicoides*) *nubeculosus* Meigen and *Culicoides* (*Monoculicoides*) *sonorensis* Wirth and Jones, are vital in increasing our understanding of the wider epidemiology of arbovirus transmission (Jones and Foster, 1978). In most

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cases these colonies provide the only available data in many areas of research due to the aforementioned difficulties of field-based studies. In this paper we therefore provide preliminary data with the aim of producing sustainable colonies of *C. imicola* in the future that will underpin our understanding of the epidemiology of *Culicoides*-borne pathogens.

2. Materials and methods

Four down-draft, 220 V light traps equipped with 8 W UV-light tube were used to collect adult midges at the ARC-Onderstepoort Veterinary Institute, South Africa (25°29′S; 28°11′E). Traps were operated daily from 4 p.m. to 6 a.m. the following morning for three weeks in November 2005. Trapped midges were transferred to disposable unwaxed 250 ml paper cups (~250/cup), covered with fine netting and kept at 26 ± 1 °C and 85% relative humidity (Venter et al., 1998). For maintenance a cotton wool pad moistened with a 10% (w/v) sucrose solution containing antibiotics (500 IU penicillin, 500 μg streptomycin and 1.25 μg/ml of fungizone) was provided daily. This was removed 72 h post-collection and midges were fed 24 h later for approximately 30 min on defibrinated sheep blood through a chicken-skin membrane (Venter et al., 1991, 1998). During feeding, lighting in the room was dimmed to ~1% daylight (~65lux).

Following feeding, *Culicoides* were immobilized in a freezer and blood engorged *C. imicola* females were selected on a refrigerated chill table and transferred to a 250 ml unwaxed paper cup (50 females/cup). Engorged females were kept at three temperatures (20 ± 1 °C, 25 ± 1 °C and 28 ± 1 °C with a 12 + 12 h photoperiod). Two replicates were done at each temperature. To facilitate oviposition, a plastic Petri dish (diameter = 35 mm) with a double layer of filter paper on top of tamped-down moist cotton wool was provided on the bottom of the cup. This provided an even surface on which eggs could be laid and reduced the danger of wings becoming stuck to wet surfaces. On the observation of eggs, the top filter paper of the double layer was transferred to a plastic Petri dish (diameter = 100 mm) filled with 10 ml of 2% agar gel medium (Boorman, 1985) and the eggs counted. The top filter paper was replaced and the adult midges and the Petri dishes with the eggs were returned to the relavant holding temperatures.

The eggs and the larvae in the Petri dishes were observed daily using a stereomicroscope against a black background and nematodes, grown on cereal powder (Pro Nutro original—Bokomo), were provided as a food source (Boorman, 1985). Distilled water was added when the agar became dry. Pupation occurred on the surface of the agar and was recorded daily. Pupae were either kept on the agar gel medium until the adults emerged or transferred to plastic cylinders (diameter = 30 mm) containing tamped-down moist cotton with filter paper on top to prevent desiccation.

In addition 20 females were removed from cups held at 25 °C at 12 h intervals and dissected under a stereomicroscope in saline solution (0.7% NaCl). The stage of oogenesis was determined using a scheme originally defined by Linley (1965) and modified by Campbell and Kettle (1975). The total number of follicles were related to body size, assessed as the mean wing length of females from basal arculus to wing tip using an optical graticule (1 unit = 0.21 mm), by regression analysis.

3. Results

The developmental period of *C. imicola* (from initial blood-feeding to the emergence of progeny adults) was highly temperature dependent, requiring 34–56 days at 20 °C, 15–21 days at 25 °C and only 11–16 days at 28 °C (Table 1). Rearing success rate (number of progeny adults produced from a single field-caught, successfully blood-fed female), was the highest in one of the replicate held at 28 °C, achieving a three-fold increase in original numbers fed (Table 1). The other replicate at this temperature, however, produced only two progeny adult males. At 25 °C and 20 °C, mean success rates were lower than the maximum rate achieved at 28 °C, but were more consistent across replicates (Table 1).

These results were primarily determined by the fecundity of females, which was highly variable at 28 °C, with both the highest (20.6 eggs/female) and the lowest (2.4 eggs/female) values recorded at this temperature. This variation was less apparent at 25 °C and 20 °C, which had means of 6.8 and 8.4 eggs per female, respectively. Eggs hatch within one (25 °C and 28 °C) to three days (20 °C) after oviposition and mean hatching rates at 20 °C (74.1%) and 25 °C (82.4%) were superior to those recorded at 28 °C (56.1%) (Table 1).

Of all the sub-adult stages the larval stage was found to have the longest duration of up to 21 days at 20 °C, and the highest mortality rates during the trials were recorded for the larval survival rates to pupation (Table 1). Although larvae were observed to feed readily upon the nematodes provided larval survival ranging from 20.9% at 25 °C to 29.2% at 28 °C, were low at all three temperatures (Table 1).

Mean emergence rates from pupae were, again, higher at 20 °C (70.2%) and at 25 °C (70.9%) than at 28 °C (43.7%) (Table 1). A heavy bias in sex ratio towards production of males was observed at all three temperatures, varying from 1:10 (females:males) at 20 °C to 1:7 at 25 °C and 1:2 at 28 °C. Where pupae were transferred to wet filter paper inside a plastic cylinder and kept at 27–30 °C the average emergence rate of 50% was higher then for pupa kept on the gel medium. These emerged adult females were successfully blood fed and eggs were produced but the hatching rates were very low and larvae did not survive after hatching.

Dissections at 12 h intervals after blood feeding shown that yolk can already occupy up to half of the oocyte 12 h after feeding. Mature eggs were observed 60 h after feeding and after 72 h most the eggs were already fully matured. After 96 h only mature eggs were observed.

The mean number of follicles counted in each group of 20 females dissected after feeding ranged from 29.0 (±5.7) to 34.0 (±8.63). The wing length in each of these groups ranged from 0.92 (±0.07) to 0.94 mm (±0.07) and no correlation could be found between the total number of follicles counted and body size.

Table 1
Duration and survival of the sub-adult stages of *C. imicola* in agar medium at three temperatures under laboratory conditions. Two replicates were done at each temperature.

Incubation temperature (°C)	Duration in days from bloodmeal to egg (mean)	No. of eggs laid	% of egg hatch (mean)	Duration in days of egg to larva (mean)	Duration in days of larva to pupa	% pupation (mean)	Duration in days of pupa to adult	% emergence (mean)	Success rate (progeny adults produced/each female fed)	Total duration in days (mean)
20	19–27 (23)	347–492 (419.5)	66.3–81.8 (74.1)	3 (3)	11–21 (16)	16.6–40.1 (27.5)	<1–5 (<3)	64.8–72.8 (70.2)	0.7–1.7 (1.2)	34–56 (45)
25	3–5 (4)	332–349 (340.5)	73.6–91.6 (82.4)	1–2 (1.5)	9–12 (10.5)	17.8–24.5 (20.9)	2–3 (2.5)	64.8–76.2 (70.9)	0.7–1.0 (0.8)	15–21 (18)
28	2–13 (7.5)	120–1032 (576)	50.0–62.2 (56.1)	1–2 (1.5)	7–9 (8)	8.3–50.0 (29.2)	<1–4 (<2.5)	40.0–47.4 (43.7)	>0.1–3.0 (1.5)	11–16 (13.5)

4. Discussion

This is the first study that shown the success on rearing *C. imicola* under laboratory conditions, from blood fed wild female to emerged progeny adults.

The mean development period, of *C. imicola* recorded during this study compares closely with that of the 24 days at 21–24 °C previously obtained by Nevill (1967). This places *C. imicola* among other anautogenous *Culicoides* species with a relatively rapid lifecycle (e.g. *C. variipennis* (*sonorensis*): Jones, 1957; *Culicoides* (Unplaced) *guttipennis*: Hair and Turner, 1966; *Culicoides* (*Oecacta*) *furens*: Linley, 1968). Fecundity, however, was low in comparison with other studies carried out on this species (e.g. 69 eggs/female; Nevill, 1967; 53–65 eggs/female: Braverman and Linley, 1994). This low fecundity could have many underlying reasons (e.g. feeding method and type and condition of blood employed), however, it was interesting to note that a mean of over 30 mature follicles/female was recorded via dissection during oogenesis, in females that were fed and kept under near identical conditions. This could imply that eggs were re-absorbed by females in the absence of a suitable oviposition substrate, indicating that an understanding of the cues for oviposition could improve this yield (Carpenter et al., 2001). Hatching rates from eggs recorded at the lower temperatures were similar to those recorded with *Culicoides* (Unplaced) *subimmaculatus* Lee and Reye (80.5%; Edwards, 1982) and *Culicoides* (*Beltranmyia*) *mississipiensis* Hoffman (72%; Davis et al., 1983). The lowest hatching rates recorded at 28 °C (56.1%) was anecdotally caused by difficulties in maintaining damp conditions for eggs.

Despite the observation that larvae of *C. imicola* fed upon nematodes provided, a relatively low proportion of these developed successfully to pupation at all temperatures. This could have been due to the density at which larvae were maintained via cannibalism or competition for resources. Although cannibalism was not observed in the current trials it is documented for other South African *Culicoides* species of e.g. *Culicoides* (*Hoffmania*) *milnei* Austen and *Culicoides* (*Beltranmyia*) *nivosus* de Meillon (Nevill, 1967). A contributory factor to the low number of larvae surviving to the pupal stage may also be that the pupa of *C. imicola* prefer relative dry habitats for pupation. Different to the larva of *C. imicola* and the pupa of most other *Culicoides* species the pupa of *C. imicola* drown on immersion in water (Nevill, 1967). Hence, gradual drying of the larval habitat, which will promote pupation, could be included in future studies.

The underlying cause for the bias towards production of males from emerged pupae is at present unknown, but is likely to be related either to higher mortality among female larvae (through a longer developmental time or other factors) or to an underlying temperature-based determination of sex (a possibility given the anecdotal observation of protandry in this species). This may indicate that the larval medium and feeding methods used are insufficient for the development of females and only allow males, with a normally shorter development period (Nevill, 1967), to develop successfully. It will, however, preclude large scale development of *C. imicola* at lower temperatures until the underlying reasons for this phenomenon are addressed.

Although this study demonstrates that it is possible to rear *C. imicola* under artificial conditions and provides essential information on the influence of temperature on the life cycle of *C. imicola*, it also highlights the many difficulties in establishing a colony from field collected material. Future studies should be aimed at determining and optimising oviposition substrate, larval medium and diet as well as the vital issue of defining successful mating conditions. Currently, this latter issue remains the most significant barrier to colonisation attempts, however, the optimised scheme developed in this paper will allow the production of the number of individuals required in order to examine this process in more detail. It would also be interesting to carry out the same study we have described here not only under artificial condition but also in natural condition in order to assess if there are significant differences and their level of variability.

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