Sampling Bias in Determining the Parous Rate of Collections of *Culicoides brevitarsis* Kieffer and *C. wadai* Kitaoka (Diptera: Ceratopogonidae)

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ABSTRACT *Culicoides brevitarsis* and *C. wadai* were collected using two updraught light traps which were run simultaneously for 107 nights. One trap, termed +cattle, was set next to a pen containing 10 cattle. The other trap, termed −cattle, was set 40 m away from the pen. Sweep-net collections of both species were also made. The parous rates of *C. wadai* from −cattle and +cattle collections and from +cattle and sweep-net collections were similar suggesting that light traps provide collections that are as representative of the biting population as that provided by sweep-net collections and that the proximity of cattle to the light trap has little effect on the parous rate of collections. This means that light traps are suitable for use in survival rate studies of this species and are not affected by the proximity of cattle. For *C. brevitarsis*, however, −cattle collections had higher parous rates than +cattle collections which in turn had higher parous rates than sweep-net collections of this species. This suggests that light traps are prone to bias and that estimates of survival of *C. brevitarsis* based on the parous rate of light-trap collections are likely to be more accurate when traps are placed in close proximity to cattle. The mean parous rate of *C. brevitarsis* from light-trap collections in the presence of cattle (0.461) was significantly higher ($P < 0.01$) than that of *C. wadai* (0.313), suggesting that the former species has a higher rate of survival and is therefore likely to be a more important vector than the latter.

Introduction

Survival of the vector is crucial in the epidemiology of vector-borne diseases and can therefore be used to compare the relative efficiency of different species of vector. A commonly used method of estimating the survival of vectors in the field is the mean parous rate proposed by Davidson (1954, cited by Clements and Patterson 1981). This rate is calculated from nightly samples and may be calculated over any number of nights. It estimates the survival of a female insect between successive blood meals, a period termed the gonotrophic cycle. A disadvantage of this method is that it requires samples that are representative of the population age-structure, particularly of the nulliparous and parous portions. Another method that has been used successfully is the time-series analysis of Birley and Rajagopalan (1981). Unfortunately this method is also sensitive to sampling bias as it requires samples that are representative of both the population size and age-structure.

Mullens (1985) reviewed the problems of sampling bias in studies on the survival of *Culicoides variipennis* Coquillett. He concluded that at least four factors can bias the composition of collections of this species, these being trap type, time of sampling, location of the trap relative to the breeding site and the presence of hosts in the vicinity of the trap. Little is known of the bias associated with collecting *C. brevitarsis* Kieffer and *C. wadai* Kitaoka, two suspected vectors of bluetongue virus in Australia, although some of the sources of bias outlined by Mullens (1985) in collecting *C. variipennis* may also affect collections of these two species.

Light traps and nets are the two most commonly used methods of collecting *C. brevitarsis* and *C. wadai* (Murray 1975, 1987, 1991; Campbell and Kettle 1979a; Kay and Lennon 1982; Standfast *et al.* 1985) and both of these methods could be subject to the sources of bias mentioned above. Light-trap collections are sensitive to differences in the attractiveness of the light to the different portions of the population, whereas net collections are sensitive to the time of collection as different portions of the population may be active at different times. Proximity of breeding sites is unlikely to be an important factor in the collection of dung-breeding species such as *C. brevitarsis* and *C. wadai*, as cattle dung is usually distributed throughout paddocks where cattle graze. Proximity of hosts, however, could affect the composition of collections made by both light traps and nets with collections made using hosts as attractants being more likely to represent what is biting the animals (i.e. nulliparous and parous females) than collections made without the use of hosts.

In addition to the four factors outlined by Mullens (1985), the size of a collection can also affect its ability to represent the population. Collections containing few specimens are more prone to inaccurate estimates of the parous rate than larger collections as the small collections may not contain representatives of one of the two groups used in the calculation of this rate. Murray (1991) proposed that some of the spurious values of the parous rate in a series of daily collections of *C. brevitarsis* were due to collections containing fewer than 20 females. He subsequently omitted these small collections from his analysis, but did not justify his decision of a minimum collection size of 20 females.

This paper investigates sources of sampling bias when estimating the parous rate of *C. brevitarsis* and *C. wadai* from light-trap collections. Compositions of light-trap collections of *C. brevitarsis* and *C. wadai* made near cattle and away from cattle were compared. The composition of these species in sweep-net collections made over cattle was also compared with light-trap collections made near cattle. These comparisons provided an assessment of sample bias present in different collections. The relative rates of survival of *C. brevitarsis* and *C. wadai* were then compared.

Materials and methods

Study site. The study was conducted at the Queensland Department of Primary Industries' Utchee Creek Research Station (17°36S 146°00E) in the seasonally wet tropics of northern Queensland. The 22-year annual average rainfall on the station is 3,600 mm, 73% of which falls between January and May.

Most of the Utchee Creek Research Station has been cleared for pasture, but remnants of rainforest persist along the creeks and hilltops. A 6 by 8 m pen was constructed along the edge of a 1 ha paddock about 15 m from a 100 m wide strip of rainforest that borders a creek running through the property. Ten cattle were run in this paddock and were placed into the pen on each night that *Culicoides* spp. collections were made.

Collection of *Culicoides* spp. Updraught light traps similar to those of du Toit (1944) but powered by a 240 Volt AC to 12 Volt DC transformer connected to mains power were used to collect *Culicoides* spp. The light traps were run in two separate locations and were termed +cattle and −cattle according to their proximity to cattle. The +cattle trap was a trap chosen randomly from six traps set around the pen containing the 10 cattle, the other five being used to supply live insects for other studies. Collections used in this study always came from the same trap. The −cattle trap was set 40 m from the pen. Because of the extra resistance offered by the 40 m lead required to connect this trap to the power source, it received only 75% of the power that the +cattle trap received. Consequently the −cattle trap's fan was slower, but the light emitted from the trap was not appreciably different from that of the +cattle trap. The traps were run from approximately 1 h before sunset to approximately 1 h after dawn on each day for 107 d from 24 January to 9 May 1991. Traps were emptied each morning.

Collections were also obtained by sweeping a conical net with an opening of 0.113m^2 over the backs of the penned cattle for a period of approximately 30 min. Collections started about 30 min after dusk or 60 min before dawn. Sweep-net collections were made on eight evenings and seven mornings. As both *C. brevitarsis* and *C. wadai* exhibit the great majority of their flight activity around cattle during these periods (Campbell and Kettle 1979b; Bellis unpublished data), it is likely that combining collections made at dusk with those made at dawn on the following morning would provide the equivalent of a nightly collection for both species. Data from these combined collections were used in subsequent analysis.

All collections were chilled at −20°C for 10 min then immersed in a normal saline solution containing 0.5% of detergent. Collections were stored at 4°C until they were sorted. Light-trap collections comprised four separate groups: the +cattle and −cattle collections of both *C. wadai* and *C. brevitarsis*. *Culicoides* spp. in the collections were sorted into species. Females were categorised according to the method of Birley and Boorman (1982) as nulliparous, blood fed nulliparous, empty parous, blood fed parous, pre-gravid and gravid according to their degree of abdominal pigmentation (Dyce 1969) and abdominal contents.

Statistical analysis. The parous rate of each daily collection from each of the four light-trap collection groups and the sweep-net collections was calculated using the formula proposed by Davidson (1954, cited by Clements and Patterson 1981):

Parous Rate = (total parous) / (total nulliparous + total parous), where
total parous = empty parous + bloodfed parous, and
total nulliparous = empty nulliparous + bloodfed nulliparous.

For each species, the parous rate of the +cattle light-trap collections was compared with the parous rate of the −cattle collections to ascertain the effect on the parous rate of proximity of cattle to the trap. Similarly, the parous rate of the sweep-net collections was compared with the parous rate of the +cattle light-trap collections. For this latter comparison, only light trap collections made during the same 7-d period as the sweep-net collections were considered. The mean parous rate of *C. brevitarsis* and *C. wadai* from +cattle light-trap collections were also compared. Comparisons were performed by fitting Generalised Linear Models (GLMs) (McCullagh and Nelder 1983) assuming a binomial error distribution and including factors for "day" (the day of collection) and "treatment" (the comparison of interest). The data exhibited extrabinomial variation (i.e. the residual variability was larger than would be expected if the data were indeed binomially distributed), so the GENSTAT 5 (GENSTAT 5 Committee 1994) library procedure EXTRABINOMIAL (Ridout and Goedhart 1993) was used.

An attempt was made to use the time-series analysis of Birley and Rajagopalan (1981) on the data generated from the −cattle light traps. Unfortunately, these data were unsuitable for the analysis as there were many occasions on which the number of parous females exceeded the number of nulliparous plus parous females collected on the previous five nights, thus violating an assumption implicit in the method.

Results

The +cattle trap consistently caught more midges than the −cattle trap.

The parous rate of *C. wadai* was not significantly different ($P > 0.10$) between +cattle and −cattle collections (0.304 and 0.334 respectively, residual df = 88). Similarly, the parous rates of sweep-net collections of *C. wadai* were not significantly different ($P > 0.10$) from those of +cattle light-trap collections (0.229 and 0.262 respectively, residual df = 6). The parous rates of +cattle collections in these two comparisons differ due to the different number of collections used in the comparisons.

The parous rate of *C. brevitarsis* was higher ($P < 0.01$) in −cattle collections than in +cattle collections (0.518 and 0.456 respectively, residual df = 90). Sweep-net collections of *C. brevitarsis* had significantly lower ($P < 0.05$) parous rates than +cattle light-trap collections (0.384 and 0.515 respectively, residual df = 6).

The mean parous rate of *C. brevitarsis* from +cattle light-trap collections was significantly higher ($P < 0.01$) than that of +cattle *C. wadai* collections (0.461 and 0.313 respectively, residual df = 106).

Discussion

It is not surprising that the +cattle trap collected more insects than the −cattle trap as cattle are a recognised host of both *C. brevitarsis* and *C. wadai* (Muller and Murray 1977; Muller *et al.* 1981). Murray (1987) and Dyce and Standfast (1979) also noted an aggregation of these species around cattle. However, the reduced power supplied to the −cattle trap and its consequently slower fan may also have contributed to this difference.

Collections containing small numbers of specimens pose a problem as they are likely to give inaccurate estimates of the parous rate. Murray (1991) dealt with this by excluding collections with fewer than 20 specimens. However, this solution introduces its own possible bias and is sensitive to the choice of minimum allowable collection size. Our use of GLMs in analysing the data, overcomes this concern as it inherently assigns a weight to each collection giving a higher weight to larger collections.

The similarity between parous rates of +cattle light trap and sweep-net collections of *C. wadai* suggests that light traps are equally attractive to nulliparous and parous females and are therefore suitable for use in studies of survival where the proportions of these age-categories are compared. Similarly, the proximity of cattle to light traps does not appear to bias the proportions of these age-categories in collections as the parous rate of +cattle and −cattle collections did not differ.

While parous and nulliparous *C. wadai* responded similarly to light traps, parous and nulliparous *C. brevitarsis* did not. Sweep-net collections had lower parous rates than the +cattle light trap. This suggests that the light trap either undersampled the nulliparous portion or oversampled the parous portion of the population. Alternatively, parous *C. brevitarsis* may not have been active during the net-sampling periods of dawn and dusk and were therefore not represented in the net collections. This latter possibility seems unlikely, as Campbell and Kettle (1979b) observed that activity of *C. brevitarsis* was relatively low during the period from after dusk to just prior to dawn compared with activity at dusk and dawn. The higher parous rate of −cattle collections compared with +cattle collections suggests that proximity of the trap to cattle can also bias collections.

The bias associated with light-trap collections of *C. brevitarsis* confounds the comparison of the mean parous rate between this species and *C. wadai.* Light-trap collections may overestimate the parous rate of *C. brevitarsis*, thereby exaggerating the differences observed between these two species.

Parous rates provide an estimate of survival over one gonotrophic cycle. In order to use parous rates to compare the survival of two species, the relative length of this cycle for both species must be known. Bellis (1993) observed that *C. brevitarsis* and *C. wadai* took a similar amount of time to digest a blood meal at 28 °C. As digestion of the blood meal is a major portion of the gonotrophic cycle, it seems reasonable to assume that these two species would also have gonotrophic cycles of similar length. Therefore the parous rates of these two species are directly proportional to relative rates of survival suggesting that *C. brevitarsis* has a higher rate of survival than *C. wadai.*

Vector survival is an important factor in the epidemiology of arthropod borne diseases, but it is only one of several important factors. This study suggests that *C. brevitarsis* has a higher survival rate and may therefore be a better vector than *C. wadai*. However, the other factors need to be taken into account when comparing vector species.

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