

Quantifying the spatial dependence of *Culicoides* midge samples collected by Onderstepoort-type blacklight traps: an experimental approach to infer the range of attraction of light traps

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Abstract. The emergence of bluetongue disease in Europe has led several countries to rapidly establish large-scale entomological surveys of its vectors, which are midges belonging to the genus *Culicoides* Latreille, 1809 (Diptera: Ceratopogonidae). These surveys have largely been based on the use of Onderstepoort-type blacklight traps. However, the range of attraction of the traps and the spatial dependence of the samples they provide are unknown, which somewhat complicates subsequent analyses. This paper investigates spatial interaction between Onderstepoort-type blacklight traps based on catches at a central trap placed close to two traps set in consecutive on/off modes. The spatial interaction is inferred from the drop in the number of midges collected in the central trap when nearby traps positioned at 50 m, 100 m or 200 m are turned on. The results showed a significant spatial interaction between traps separated by 50 m for female *Culicoides obsoletus*/*Culicoides scoticus* and *Culicoides dewulfi*. No significant interaction was found for female *Culicoides* of other species, for male *Culicoides*, or for traps spaced at ≥ 100 m. Based on the experimental design geometry and on simple assumptions on the distribution of *Culicoides* midges in the neighbourhood of the traps, the paper also presents a method to infer the range of attraction of the traps.

Key words. Biting midges, bluetongue disease, landscape-scale distribution, light trap, sampling method, vector distribution.

Introduction

Bluetongue (BT) has been reported as one of the main vector-borne diseases of livestock in Europe (Wilson & Mellor, 2008), especially since 2007, when it spread rapidly in northwestern parts of Europe, where it is now considered endemic (e.g. Saegerman *et al.*, 2008, 2010; European Commission, 2010). The disease is transmitted by the females of several species of midge belonging to the genus *Culicoides* (Mellor *et al.*, 2000). In the absence of BT, measuring the presence or absence of vectors in an area is a priority as it helps to quantify the risk for local establishment of the disease. By contrast,

when BT is endemic, longitudinal surveys of the vectors allow for the determination of vector-free periods that can be used to partially relax restrictions on livestock movements between countries (Carpenter *et al.*, 2009). Furthermore, vector abundances and occurrence data provided by *Culicoides* sampling can also be used to establish biting rates. This estimate is needed to establish BT basic reproduction number (R₀) (Gubbins *et al.*, 2008; Raclouz *et al.*, 2008; Hartemink *et al.*, 2009), a metric used to predict the status of an epidemic and to allow the modelling of the spread of BT within and between farms (Szmaragd *et al.*, 2009).

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Characterizing *Culicoides* populations requires a standardized method of sampling so that results obtained from different sites at different times can be compared. Many studies have based their sampling on light trap collections (Dyce *et al.*, 1972; Bishop *et al.*, 2000; Pili *et al.*, 2006) and more specifically on the use of Onderstepoort blacklight traps. These have been used for over 10 years in Europe (e.g. Calistri *et al.*, 2003; Conte *et al.*, 2003, 2009; Purse *et al.*, 2004; De Deken *et al.*, 2008; Patakakis *et al.*, 2009; Nielsen *et al.*, 2010) and have become the recommended standard method of sampling for *Culicoides* specimens (Mellor *et al.*, 2004). However, there is a general debate on the use of light traps and the artificial responses they induce in flying insects (Blomberg *et al.*, 1976; Frank & Case, 1988; Southwood & Henderson, 2000) that makes the interpretation of catches difficult (Braverman & Linley, 1993; Venter *et al.*, 2009). More specifically, the validity of inferring *Culicoides* abundance and composition from the catches of Onderstepoort blacklight traps was recently questioned (e.g. Carpenter *et al.*, 2008; Gerry *et al.*, 2009) because of a number of uncertainties about how well the catches represent the actual local population. The range of attraction of the traps and the spatial dependence of collected samples are important unknowns that should be characterized for three main reasons. Firstly, this information would assist in the interpretation of surveillance data as the actual area of the site sampled would be better known. Secondly, it would allow for inference of local densities of vectors from trap data (e.g. Hartemink *et al.*, 2009). Thirdly, in conditions in which Onderstepoort blacklight traps are used to replicate sampling in a series of locations, such as in landscape-scale studies (Guis, 2007), knowledge of such unknowns would allow for the setting up of experimental designs that ensure spatial independence between samples.

This paper presents a turn-off/turn-on method that aimed to quantify the spatial interactions between Onderstepoort blacklight traps separated by increasing distances. As a measure of spatial interaction, the method quantifies the change in catches in a central trap as a function of the status (On or Off) of two nearby traps. Based on these results, simple assumptions and geometric relationships, a method to infer the range of attraction of Onderstepoort blacklight traps for species of the genus *Culicoides* is described.

Materials and methods

Study area

The experiments were conducted in June and July 2009 in a pasture on a dairy farm located in Belgium (50°49'38.71" N, 04°15'43.61" E; 28 m a.s.l.) (Fig. 1). Cattle were either present or absent from the pasture during the day, but were not present during the nights of sampling. A portable meteorological station (Ventage Pro 2; Davis Instruments Corp., Hayward, CA, U.S.A.) was set in the pasture as soon as the handling began. This station recorded temperature (min/max), rainfall, relative humidity, atmospheric pressure, dew point, and wind intensity and direction, at 15-min intervals. Particular attention was paid to ensuring the experiments were conducted only when



Fig. 1. Study site and the various types of land use on the farm. White areas indicate areas close to farm buildings; permanent host shelter is indicated by the left rectangle. Light and medium grey areas indicate, respectively, crop and pasture. Dark grey areas indicate mixed areas (mainly crops, roads and houses). ●, traps; *, meteorological station. Trap iv is visually isolated from traps i, ii and iii by a permanent wall and relief (illustrated by the white line). Distances between i and ii or iii may vary (see Fig. 2).

moonlight was constant and not too bright (i.e. around the new moon or, if at other times, when nights were clear and cloudless, but never during a full moon) [moonlight has been found to interfere with *Culicoides* sampling (Bowden, 1973; Bishop *et al.* 2000)]. Other artificial sources of light were noted, but all were located far from the pasture and appeared to be of constant intensity throughout the experiment.

Experimental design

Four new Onderstepoort-type blacklight traps [Agricultural Research Council, Onderstepoort Veterinary Institute (ARC-OVI), Pretoria, South Africa] supplied with 12-V calcium batteries (44 Ah, 210 A), DC/AC power inverters (100 W) and time programmers (EMT757-F; Chacon SA, Wavre, Belgium) were used to catch the insects. They were positioned within a pasture at a constant height of 2.2 m and fixed onto 3.5-m wooden masts pushed into the ground.

The principle of the turn-off/turn-on design is that it allows for measurement of the difference in the number of insects caught in an Onderstepoort blacklight trap when two nearby traps are alternately switched on and off. More specifically, light traps were positioned as indicated in Figs 1 and 2: a central trap (trap i) was set up in the middle of the pasture and two additional traps (traps ii and iii) were positioned in the same pasture at a distance d from the central trap. A fourth trap (trap iv) was placed in a control area out of direct sight of the three other traps; catches at trap iv were carried out continuously over the sampling period. Ideally, a control should be identical in all conditions except that under study. Therefore, as this study aimed to specifically quantify the effect of spatial interactions, the control trap had to be placed far enough from the other three traps to avoid it being influenced by any of them. However, placing the control trap too far from the

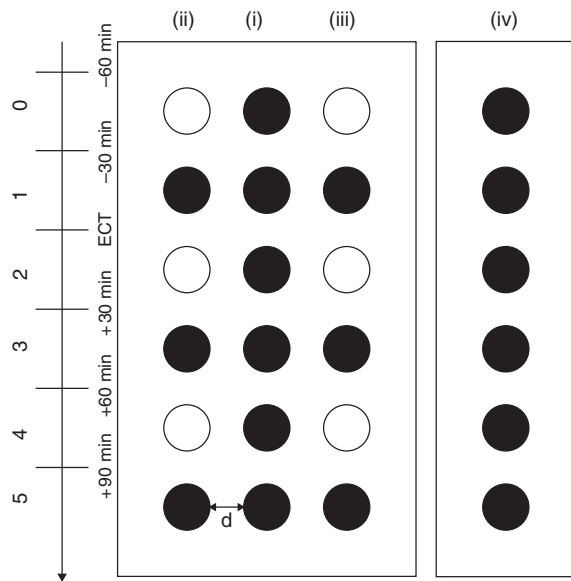


Fig. 2. Turn-off/turn-on design from 1 h before the end of civil twilight (ECT) until 2 h 15 min after ECT (six time periods). Nearby traps (ii and iii) were turned alternately on (●) and off (○) every 30 min (+3 min for manual collection). Central (i) and control (iv) traps were emptied simultaneously every 30 min.

others may have resulted in catches obtained in very different local conditions. Alternatively, masking it from the view of the other traps may also have influenced the catches. The fourth trap was thus positioned near the cattle shed, but hidden from the other traps. Although the placement of the control trap may have introduced a difference in trapping conditions compared with the three traps located in the pasture, it allowed us to compare our study catches with those obtained using standardized sampling protocols (Goffredo & Meiswinkel, 2004).

The experiment involved collecting insects in both the central (i) and control (iv) traps while the nearby traps (ii and iii) were alternately turned off and on every 30 min. Trapping started 1 h before the end of civil twilight (ECT) and ended 2 h and 15 min after ECT and was divided into six 30-min periods. In order to permit the handling of samples, the central and control traps were switched off for 3 min following each sampling period. Three distances (d) between traps were tested: 50 m, 100 m and 200 m. For each distance, the experiment was repeated during three consecutive nights, with turn-off/turn-on sequences as follows: night 1: Off/On/Off/On/Off/On (Fig. 2); night 2: On/Off/On/Off/On/Off, and night 3: Off/On/Off/On/Off/On.

Sample identification

Insects were collected in absolute ethyl alcohol (AnalaR Normapur®; VWR International BVBA, Leuven, Belgium) and brought to the laboratory to be sorted. When visual examination of the sample suggested that the total number of insects caught exceeded 5000 individuals, subsamples were taken following a modified protocol based on Van Ark &

Meiswinkel (1992) and Goffredo & Meiswinkel (2004): the sample was diluted in a graduated tube (BD Falcon™; BD Biosciences, Inc., Billerica, MA, U.S.A.) and, depending on the level of insects in the tube, alcohol was added to 20 mL, 25 mL, 30 mL, 37.5 mL or 40 mL. Four or five replicated samples of 2.5 mL were then taken from the tube and, respectively, determined under stereomicroscope. The number of insects was then extrapolated by multiplying the sum of all four or five replicates by their level of dilution. Females and males belonging to the genus *Culicoides* were first separated from remaining insects (mainly Ceratopogonidae and Psychodidae) based on typical wing patterns and general shape (Delécolle, 1985; Goffredo & Meiswinkel, 2004). Females were sorted to species level according to the numerical key of Mathieu *et al.* (2010) and, if possible, were age-graded according to their abdominal pigmentation (Dyce, 1969). Finally, some species were grouped into their respective subgenera (Delécolle, 1983) prior to the analyses, for three reasons. Firstly, there is high variability in the morphological criteria used to differentiate some species (e.g. wing patterns of *Culicoides circumscriptus* and *Culicoides salinarius* in the subgenus *Beltranmyia*). Secondly, mounting specimens for observations under an optical microscope is time-consuming (e.g. to differentiate *Culicoides achrayi* from *Culicoides pallidicornis* or *Culicoides subfascipennis* within the subgenus *Sylvaticulicoides*). Thirdly, analysing data for species in which specimens were collected in low numbers would have made the use of any parametric statistic difficult.

Within-night patterns

In order to quantify the deviation between the numbers of insects collected in the central trap when the nearby traps were turned off or on, temporal variations within and between consecutive nights of capture had to be taken into account. Because of the high degree of inter-night variability in *Culicoides* catches, a reference model was applied to within-night control trap catches to control for the variations encountered during the experiment's 9 nights of collection. Prior to parameter fitting, data were log-transformed to minimize dependence of the variance upon the mean and grouped in a reference night divided into six time periods. The data were fit to a quadratic regression model as a function of time (t) (Eqn 1). This model was chosen for three reasons: (a) to account for the expected bell-shaped curve of adult flight activities that may occur at dusk (Service, 1971); (b) to allow the initial population level to differ from zero, and (c) to facilitate the estimation of parameters with the linear regression in R (R Development Core Team, 2010):

$$y = \alpha t^2 + \beta t + \gamma \quad (1)$$

Using mean values and standard deviations of the parameters provided by the quadratic regression, and assuming that parameters were normally distributed, a 95% confidence interval (CI) was constructed by sampling each parameter 500 times. Normality of residuals and homoscedasticity were controlled as recommended in Venables & Ripley (2002) and Ruxton & Beauchamp (2008).

Spatial dependence

Data collected for the central trap were corrected by calculating the difference between each observation and a value sampled for the same time period from the reference model 95% CI. The difference δ between values in On and Off conditions was then assessed for each distance. The mean difference δ obtained for each distance was compared with zero using a one-sample *t*-test. Given the multiple comparisons required for all three distances, a Bonferroni correction was used to establish whether differences differed significantly from zero.

Range of attraction

According to Southwood & Henderson (2000), the number of individuals of a given taxonomic group in a fixed physiological state [phase (Φ) ~ 1] caught in a particular light trap with an assumed constant light intensity is a function of the number of insects in the studied habitat multiplied by a given probability that they respond to the light stimulus (i.e. here, \sim range of attraction). Even if *Culicoides* breeding spots observed in an area of homogeneous land use would appear to be patchily distributed, one may assume that adults will disperse in every direction immediately after taking flight (Taylor & Brown, 1972). Therefore, if hosts are absent or beyond their attraction range [see Gillies & Wilkes (1970) for a methodological approach], the insects could be assumed to be distributed at random. Under these conditions, the abundance of insects caught in the light trap (n) divided by surface covered by the light trap (s) (i.e. the distance over which these insects respond to the light stimulus) should be proportional to the density of insects in the habitat multiplied by Φ (Eqn 2):

$$n/s \propto \Phi \cdot N_{tot}/S_{tot} \quad (2)$$

If N_{tot} is assumed to be constant, and the spatial distribution in the studied habitat is assumed to be homogeneous, the measured deviations δ in the central trap when nearby traps are turned on should be proportional to the number of insects present at the intersection between the central and nearby traps (N_{int}) divided by 2 and multiplied by the number k of nearby traps (Fig. 3). The number of insects in the intersection surface S_{int} (Fig. 3, hatched area) is inferred from the drop in catches (Eqn 3):

$$N_{int} \propto \frac{2\delta}{k} \quad (3)$$

The surface of intersection S_{int} between traps can be geometrically calculated as a function of the attraction radii r (considered identical for all traps) and of the inter-trap distances d according to (Eqn 4):

$$S_{int} = k \left(2r^2 \arctan \left(\frac{2\Omega}{d} \right) - d\Omega \right), \quad (4)$$

$$\text{where } \Omega = \sqrt{r^2 - \left(\frac{d}{2} \right)^2}$$

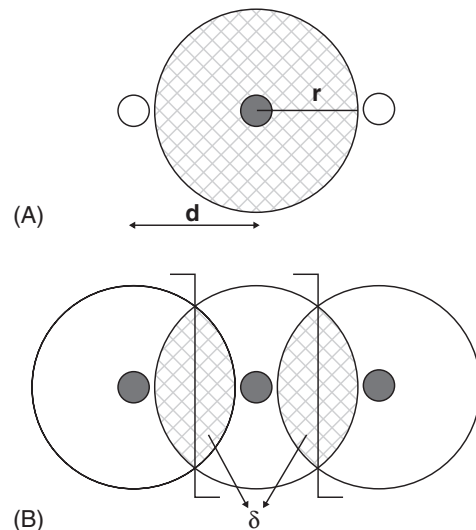


Fig. 3. Diagram of the interactions that might occur between the central and two nearby traps, in (A) Off mode and (B) On mode. Areas in which the density of insects was inferred are hatched. The δ -value is measured according to the deviations between catches in the central trap when the nearby traps are Off and On. Theoretically, δ corresponds to N_{int} divided by twice the number of nearby traps.

By combining Eqns (3) and (4), the insect density in the intersection surface $D_{int} \sim N_{int}/S_{int}$ can be expressed as a function of δ , r and d . In Off conditions, D_{off} is proportional to N_{off}/S_{off} , where N_{off} is the number of insects caught in the central trap (estimated by the arithmetic mean of the accumulated number of *Culicoides* calculated for every replicate) and S_{off} is the surface of a circle of radius r .

Finally, the attraction range of the Onderstepoort blacklight trap can be estimated as the radius r satisfying the conditions $D_{int} = D_{off}$. Uncertainties can be estimated by the shared surface of the 95% CIs for the curves of inferred densities D_{int} and D_{off} .

Results

A total of 39 491 individuals belonging to the genus *Culicoides* were sampled during the 54 collections. Among these, 41% and 54% were females caught in the central and control traps, respectively. The remaining 5% were identified as males. Females collected during the first 36 collections were identified to species level and graded by age (Table 1). Most of them belonged to the *Avaritia* subgenus (60% in the central trap and 90% in the control trap), which was mainly represented by females of *Culicoides obsoletus/Culicoides scoticus* and *Culicoides dewulfi*. There was no significant difference in the ratio of parous : nulliparous insects ($F_{4,283} = 0.1$, $P > 0.05$). A summary of the weather conditions that occurred during 21.45–01.00 hours on each night of the sampling period is provided in Fig. S1 (online). No rain fell during the sampling period. All other parameters were within normal ranges for the season.

Table 1. Numbers of nulliparous and parous female *Culicoides* collected during trapping nights with secondary traps placed at 50 m and 100 m.

Total female <i>Culicoides</i>	Control trap catches (<i>n</i> = 36)		Central trap catches (<i>n</i> = 36)*			
	Nulliparous	Parous	Off-mode		On-mode	
			Nulliparous	Parous	Nulliparous	Parous
<i>Avaritia</i>		14 417		4217		2919
<i>Obsoletus</i> complex	28%	26%	1845	1004	1230	858
<i>C. chiopterus</i>	<1%	5%	95	495	21	294
<i>C. dewulfi</i>	21%	19%	287	491	269	247
<i>Culicoides</i>		698		881		617
<i>C. pulicaris/C. lupicaris</i>	6%	11%	50	41	38	21
<i>C. punctatus</i>	20%	54%	292	435	131	373
<i>C. newsteadi</i>	3%	3%	16	42	13	33
<i>Beltranmyia</i>		177		306		110
<i>C. salinarius/C. circumscriptus</i>	68%	31%	87	195	56	50
<i>Monoculicoides</i>		316		117		107
<i>C. nubeculosus/C. puncticollis</i>	2%	63%	4	57	3	69
<i>C. riethi</i>	9%	14%	34	19	1	23
<i>C. stigma/C. parroti</i>	4%	7%	0	3	0	11
<i>Sylvaticulicoides</i>		214		1027		683
<i>C. picturatus</i>	11%	71%	146	693	52	449
<i>C. pallidicornis</i>	2%	5%	2	128	12	63
<i>C. subfascipennis</i>	1%	1%	2	21	4	19
<i>C. achrayi</i>	1%	4%	8	13	20	50
Other subgenera		190		544		419
<i>C. kibunensis</i>	2%	98%	20	475	4	346
<i>C. festivipennis</i>	–	–	1	41	7	38
Other species†	–	–	6	0	2	22
Total		16 012		7 092		4 855

*Specimens for which physiological status was not determined were added to the total numbers for each subgenus.

†*Culicoides albicans*, *Culicoides pictipennis*, *Culicoides duddingstoni*, *Culicoides poperinghensis*, *Culicoides tbilizicus* (first record in Belgium), *Culicoides segnis* and *Culicoides minutissimus*.

Within-night patterns

Within-night patterns of numbers collected and the reference models for female or male *Culicoides* collected in the control trap revealed the highest level of catches from around 30 min after ECT until 1 h later (Fig. 4A, B). This pattern mostly reflected the most abundant species (i.e. *C. obsoletus/C. scoticus* and *C. dewulfi*) (Fig. 4C, D). Coefficients for the reference models are summarized in Table S1. Temporal patterns for female *Culicoides chiopterus*, *Culicoides kibunensis* and females belonging to *Culicoides*, *Beltranmyia*, *Monoculicoides* and *Sylvaticulicoides* subgenera are provided in Fig. S2.

Spatial dependence

The mean deviations between the corrected numbers of *Culicoides* collected in the central trap when the nearby traps were in the Off and On conditions are plotted in Fig. 5. The deviations are plotted for the three distances tested and separately for females (Fig. 5A) and males (Fig. 5B). These deviations were significantly higher than zero for female *Culicoides* at 50 m (range of *t*-tests with $\alpha = 0.025$, $P < 0.05$), but not for males ($P > 0.05$) and never at 100 m or 200 m ($P > 0.05$). Mean deviations at 50 m were significantly higher than zero for most subgroups and species considered in Table 2, but none of them

differed significantly from zero at 100 m. Note that no significant deviations for subgroups other than *Avaritia* emerged at 50 m when they were analysed separately.

Range of attraction

Based on an inter-trap distance of 50 m, the attraction range satisfying $D_{int} = D_{off}$ was 29.6 m (95% CI 26.3–31.9) (Table 3) for all female *Culicoides* (Fig. 6). The attraction ranges estimated for female *Avaritia*, female *C. obsoletus/C. scoticus* and female *C. dewulfi* (grouped or age-graded as parous), and the group of remaining subgenera were in the same range of values (min = 25.5, max = 33.2; Table 3). The range of attraction was not estimated for the groups when the drop in catches according to On and Off conditions did not significantly differ from zero.

Discussion

The turn-off/turn-on method introduced here allowed for the quantifying of interactions between Onderstepoort blacklight traps set at different distances. The study also proposes a way to use these data to infer the attraction range of *Culicoides* midges collected in these traps. More specifically, a measurable interference between Onderstepoort blacklight

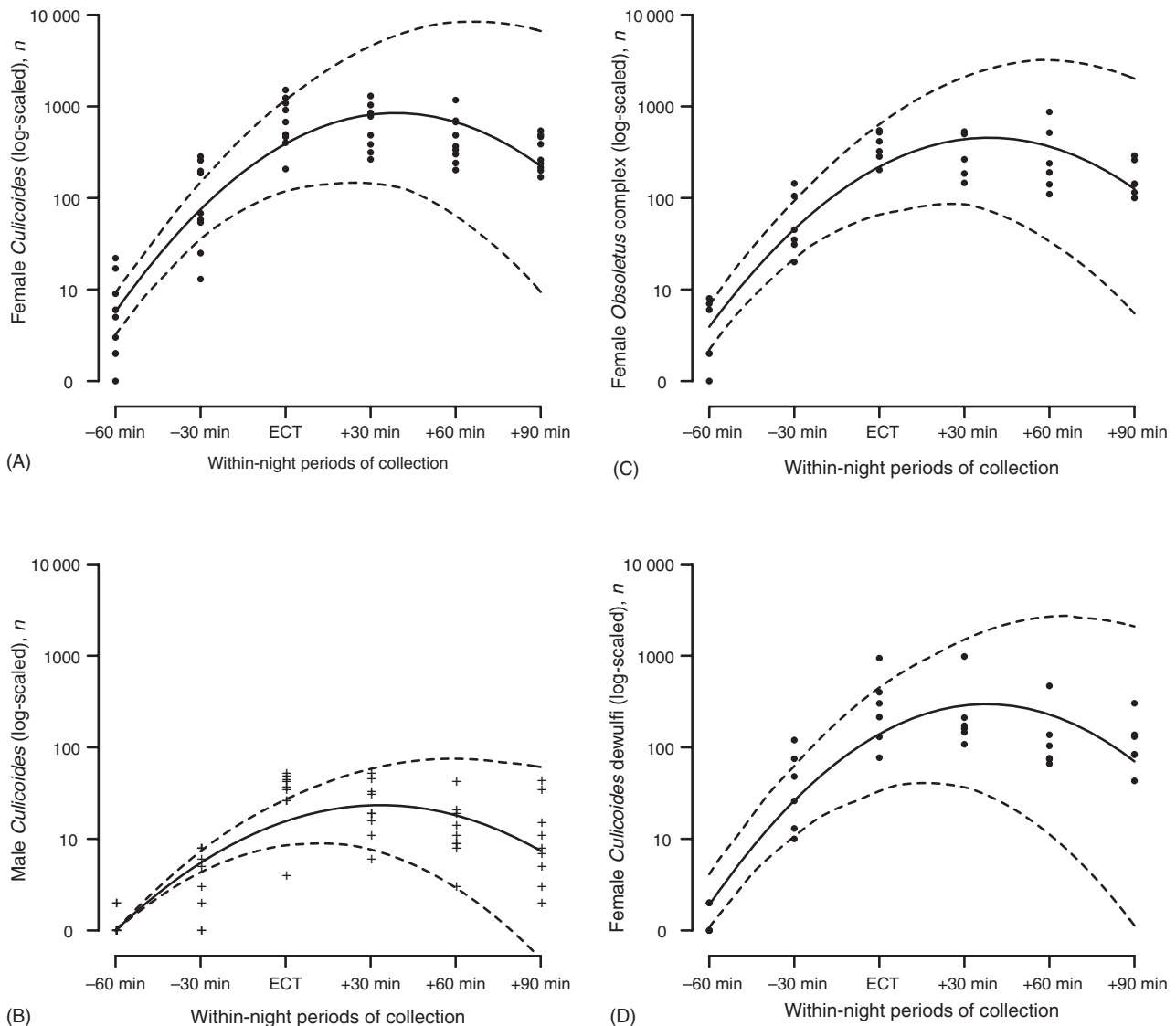


Fig. 4. Within-night flight patterns for (A) all female *Culicoides*, (B) all male *Culicoides*, (C) female *Culicoides obsoletus/Culicoides scoticus* (C) and (D) female *Culicoides dewulfi* collected in the control trap from 1 h before the end of civil twilight (ECT) until 2 h 15 min after ECT. Fitted polynomial models are shown (continuous lines) within 95% confidence intervals (dotted lines).

traps was observed when the traps were separated by distances of 50 m, but not by distances of 100 m or 200 m, and differed according to the species and physiological status of the females. Interference between traps could not be measured for males, which suggests that the interaction may occur over even shorter distances. Therefore, the range of attraction for female *Culicoides* was approximated to a value of 29.6 m, and, assuming all else is held constant, also differed according to species and physiological status. Female *Avaritia* and, more specifically, female *C. obsoletus/C. scoticus* were attracted from an area of 0.24 ha around the Onderstepoort blacklight trap. This range was estimated to be slightly higher for female *C. dewulfi* (~0.25 ha), but, given the CI of both estimates (Table 3), the pattern appears identical in both species. The range of attraction was estimated to be higher for parous female

C. dewulfi (~0.32 ha around the trap) than for any other remaining groups studied here (Table 3). This result suggests that this species, in this particular physiological state, may be able to respond to the light stimulus from a greater distance than nulliparous female *C. dewulfi* or any other species in any other physiological state. Such a conclusion, however, requires confirmation by additional studies, perhaps arising from the growing interest in studying the fine-scale ecology of BT vectors (e.g. Guis, 2007; Meiswinkel *et al.*, 2008; Takken *et al.*, 2008; Zimmer *et al.*, 2009), and aiming to better quantify and characterize vector populations in the landscape.

Several additional aspects of the method used to infer the attraction range warrant discussion.

Firstly, the time interval between On and Off conditions was fixed to 30 min; at a given flight speed, this limits the range

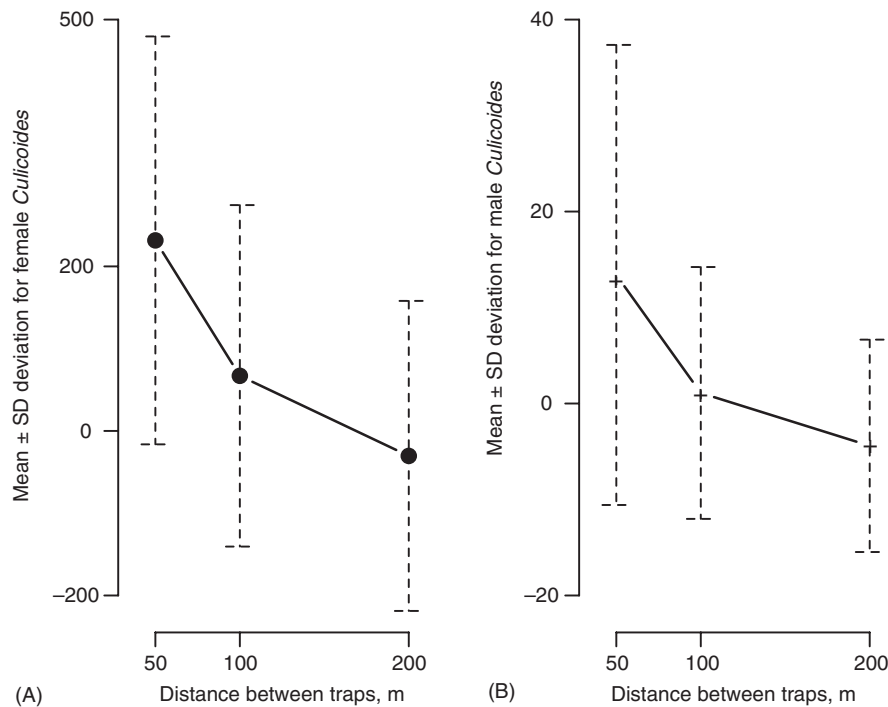


Fig. 5. Mean deviations (δ) [\pm standard deviation (SD)] between *Culicoides* numbers collected at the central trap in ‘without interference’ mode (Off-mode) and in ‘with potential interference’ mode (On-mode), for (A) females and (B) males, as a function of the distances separating the traps.

Table 2. Deviations (δ 50 and δ 100) between Off-mode and On-mode trapping sessions, at inter-trap distances of 50 m and 100 m.

Female <i>Culicoides</i>	δ 50	\pm SD	<i>P</i> -value	δ 100	\pm SD	<i>P</i> -value
<i>Avaritia</i>	65.83	94.51	<0.001	17.29	86.60	NS
<i>C. obsoletus/C. scoticus</i>	41.50	64.95	0.01	10.38	63.97	NS
<i>C. chiopterus</i>	9.40	34.62	NS	3.40	18.69	NS
<i>C. dewulfi</i>	14.88	25.93	<0.001	3.29	17.09	NS
<i>C. dewulfi</i> nulliparous	2.63	21.10	NS	1.97	17.95	NS
<i>C. dewulfi</i> parous	27.14	24.47	<0.001	4.60	16.41	NS
Other subgenera	19.80	39.39	<0.001	7.00	29.49	NS

A significant *P*-value indicates that interference occurred. SD, standard deviation; NS, not significant.

of attraction that might possibly be measured. Former studies based on mark–release–recapture experiments with *Culicoides* suggested a mean flight speed of 50–100 m/30 min during night-time activity, defined as that occurring between sunset and sunrise (Lillie *et al.*, 1981, 1985; Brenner *et al.*, 1984). However, if European species are assumed to have similar capacities, the time interval for the On and Off conditions should have allowed us to measure attraction ranges of ≥ 50 m (i.e. interaction distances > 100 m). Using a longer time interval for the On and Off conditions might permit the detection of interactions over longer distances, but would reduce the number of comparable On and Off periods over a single night of catches.

Secondly, interactions were detected over relatively short distances only, which may partially reflect a lack of statistical power. For example, the results illustrated in Fig. 5 show that the mean deviation between On and Off conditions for

Table 3. Ranges of attraction inferred for species (or groups of species) expressing significant interferences and 95% confidence intervals (CIs).

	Range of attraction	95% CI
Female <i>Culicoides</i>	29.6	26.3–31.9
<i>Avaritia</i>	27.6	25.6–29.3
<i>C. obsoletus/C. scoticus</i>	27.5	25.4–29.4
<i>C. dewulfi</i>	28.0	25.5–30.1
<i>C. dewulfi</i> parous	31.8	27.2–33.2
Other subgenera	26.4	25.5–27.8

an inter-trap distance of 100 m was > 0 , although this difference was not statistically significant. Moreover, the geometry of the experimental design is such that the higher the distance between traps compared with the range of attraction, the smaller the intersection will be in proportion to the total

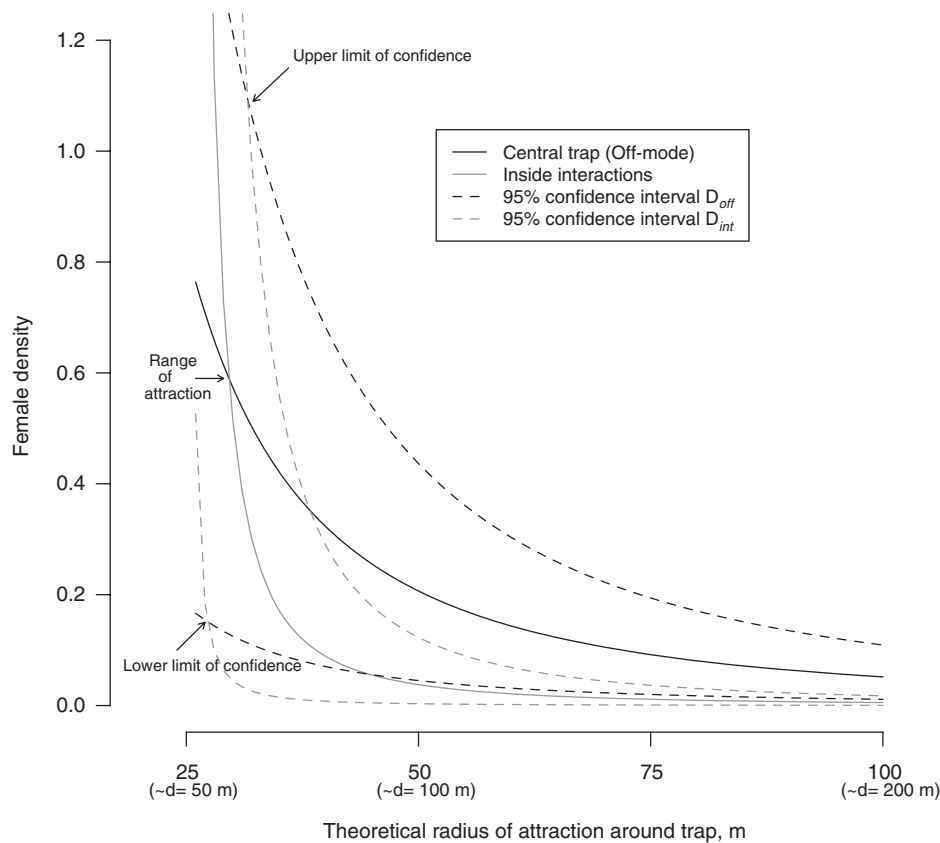


Fig. 6. Densities of female *Culicoides* (\pm 95% confidence interval) in Off-mode and at the intersection of the interacting light traps for $d = 50$ m, as a function of theoretical attraction radii.

catches, thereby reducing the chances of measuring a significant drop in catches when nearby traps are turned on. A number of options could be considered to increase the statistical power at greater distances, such as surrounding the central trap by more traps (i.e. increasing the surface of interaction between traps) or reducing the variability in trapping conditions.

Thirdly, a relatively simple model was used to infer the range of attraction from the interaction distances, and more elaborate models based on different assumptions could be developed (e.g. such a model might assume a non-random distribution of insects in the range of the trap). However, given the fairly high variability in the catches themselves, a more elaborate model would not necessarily result in a significant increase in accuracy.

A disparity in species composition was noted between the central and control traps, probably because the control trap was closer to the cattle shed than the central trap. The impact of this disparity on our results is believed to be minor because the control trap data were used only to predict the overall curve of catches as a function of time and hence to correct the catches of the central trap according to the emergence pattern and the timing of the On/Off sequence. However, in future studies, it may be advisable to set the control trap in an environment similar to that of the central trap.

Thus, despite some limitations, two important conclusions can be drawn from the study. Firstly, trapping carried out in

order to sample local populations, such as to study vector distribution in a Belgian rural landscape, can be conducted with spatial independence between traps set relatively short distances apart (provided that the duration of collection does not exceed 30 min close to ECT). Secondly, several studies have tried to relate insects collected on hosts to those collected by light traps (e.g. Carpenter *et al.*, 2008; Gerry *et al.*, 2009) and, more recently, to establish the effects of host abundance (Garcia-Saenz *et al.*, 2010), host presence (Baylis *et al.*, 2010) and larval distribution (Foxi & Delrio, 2010) on light trap samples. These studies might have benefited, indirectly or directly, from a better understanding of the potential range of attraction and interferences between Onderstepoort blacklight traps. It is clear that many local (e.g. species abundance and composition, environmental conditions) and experimental (e.g. light intensity of Onderstepoort blacklight traps) factors, not studied here, may have influenced the spatial range of catches in these other studies. Translating the results to different agro-ecological landscapes and experimental conditions is hence not straightforward. However, the present study showed that a relatively limited number of collection nights is needed to assess the spatial independence of Onderstepoort blacklight trap catches for a given distance interval, and this method might therefore be considered as a screening method to be used prior to intensive studies relying on Onderstepoort blacklight trap sampling.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: DOI: 10.1111/j.1365-2915.2011.00988.x

Figure S1. Boxplots showing mean values of temperature, relative humidity, atmospheric pressure, dew point, wind speed and wind direction recorded during each period of replication. Note that no particular wind direction was recorded in replicates 2, 3 and 1'.

Figure S2. Within-night activity patterns, fitted polynomial models and 95% confidence intervals for female *Culicoides chiopterus*, for females belonging to *Culicoides*, *Beltramyia*, *Monoculicoides* and *Sylvaticulicoides* subgenera and to remaining subgroups (mainly one species, *Culicoides kibunensis*).

Table S1. Summary of the parameters (mean \pm standard error) describing the polynomial models used to describe within-night patterns for *Avaritia* (*Culicoides obsoletus*/*Culicoides scoticus*, *Culicoides chiopterus* and *Culicoides dewulfi*) and other subgenera.

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