

Farms, pastures and woodlands: the fine-scale distribution of Palearctic *Culicoides* spp. biting midges along an agro-ecological gradient

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Abstract. The spatial epidemiology of Bluetongue virus (BTV) at the landscape level relates to the fine-scale distribution and dispersal capacities of its vectors, midges belonging to the genus *Culicoides* Latreille (Diptera: Ceratopogonidae). Although many previous researches have carried out *Culicoides* sampling on farms, little is known of the fine-scale distribution of *Culicoides* in the landscape immediately surrounding farms. The aim of this study was to gain a better understanding of *Culicoides* populations at increasing distances from typical dairy farms in north-west Europe, through the use of eight Onderstepoort-type black-light traps positioned along linear transects departing from farms, going through pastures and entering woodlands. A total of 16 902 *Culicoides* were collected in autumn 2008 and spring 2009. The majority were females, of which more than 97% were recognized as potential vectors. In pastures, we found decreasing numbers of female *Culicoides* as a function of the distance to the farm. This pattern was modelled by leptokurtic models, with parameters depending on season and species. By contrast, the low number of male *Culicoides* caught were homogeneously distributed along the transects. When transects entered woodlands, we found a higher abundance of *Culicoides* than expected considering the distance of the sampling sites to the farm, although this varied according to species.

Key words. Bluetongue disease, landscape distribution, spatial epidemiology, transect sampling, vector ecology.

Introduction

Bluetongue is a vector-borne disease affecting even-toed ungulates (Erasmus, 1975; Chandel & Kher, 1999). The role of female *Culicoides* in the transmission of the disease was described for the first time in South Africa (Du Toit, 1944). Since then, species vectoring the disease have been described in many countries (Mellor *et al.*, 2000). The descriptions most often followed intensive entomological surveillance triggered by the detection of the bluetongue virus (BTV) (e.g. in the US:

Hardy & Price, 1952; Price & Hardy, 1954; Tabachnick, 1996; and in Latin America: Lager, 2004) but sometimes even before BTV was detected (e.g. in the UK: Jennings & Mellor, 1988). The rapid geographical expansion of BTV in Europe in recent years can be linked to several factors, although the relative contribution of each of these remains uncertain: intensive trade and movements of animals (Randolph & Rogers, 2010), global environmental change (de La Rocque *et al.*, 2008; Tabachnick, 2010) and the pre-existence of indigenous competent *Culicoides* spp. vectors (Conte *et al.*, 2009).

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Over the last 10 years, large-scale entomological surveillance programmes have been carried out in many countries affected by BTV using standardized sampling methods (Calistri *et al.*, 2003; Conte *et al.*, 2003; Purse *et al.*, 2004a; De Deken *et al.*, 2008; Conte *et al.*, 2009; Patakakis *et al.*, 2009; Nielsen *et al.*, 2010). These studies have allowed better documentation of the role of several species in the distribution of BTV and quantification of the seasonal activities of the vectors (Purse *et al.*, 2006). Furthermore, they have also helped to detect vector-free periods in order to allow animal trade between regions with established infections (Carpenter *et al.*, 2009). Entomological surveillance data have also been used to train statistical models with the aim of predicting large-scale distributions of the main vectors (Conte *et al.*, 2004; Purse *et al.*, 2004b, 2007; Pili *et al.*, 2006). Standardized sampling protocols used for entomological surveillance recommend sampling within the farms or animal shelters. This allows maximization of the efficiency of the trap and sampling of populations that are close to the potentially infected hosts. By contrast, very few studies have reported sampling performed outside the farms, in the surrounding landscape (Guis, 2007; Meiswinkel *et al.*, 2008; Takken *et al.*, 2008; Zimmer *et al.*, 2009). As a consequence, little is known of the fine-scale distribution of *Culicoides* in these landscapes. More specifically, the ecological requirements of the vectors and the localization of their resting roosts (such as defined by Cheng & Hogue, 1974; in Blackwell *et al.*, 1992; or in Carpenter *et al.*, 2008) and even laying roosts (Blackwell *et al.*, 1999) remain poorly known. Sampling carried out in various habitats may also facilitate interpretation of correlations reported in previous studies between landscape metrics and the occurrence of BTV or adult vectors (Guis *et al.*, 2007a, b; Takken *et al.*, 2008). Finally, including woodlands as part of the out-of-farm sampling is also important as it may provide information on the transmission/circulation of BTV between wild ungulates.

The aim of this study was to quantify how *Culicoides* sampled through Onderstepoort-type black-light traps vary in abundance and species composition at an increasing distance from farms in the surrounding pastures, and further apart in woodlands. As *Culicoides* activity and animal husbandry are both strongly seasonal in northern Europe, the sampling was carried out in spring and autumn.

Materials and methods

Sampling sites and times

The study was conducted at five dairy farms located in the five largest ecological regions of Belgium (Fig. 1, Vanderमotten & Decroly, 1995). These farms were declared positive to BTV during the 2007 and 2008 serological surveys carried out by Belgian authorities. They were selected to be surrounded by continuous pastures over a radius ranging from 200 to 400 m connecting the farm animal shelter to the nearest forest (deciduous or mixed, with potential wild hosts present). Sampling was conducted over two periods corresponding to different farming activities. Spring is the beginning of the grazing season. Cattle, mainly of the Holstein breed, are not

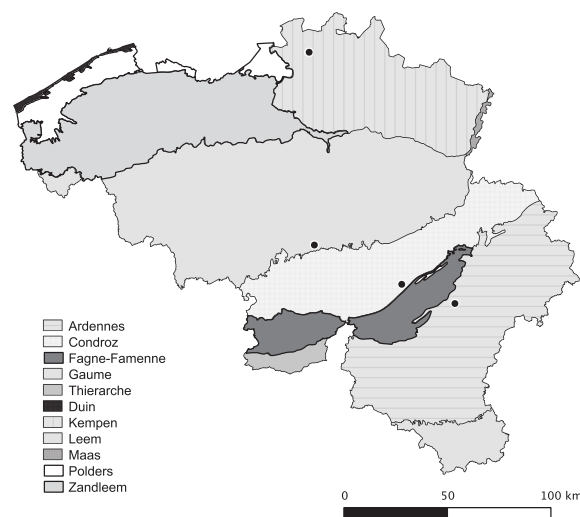


Fig. 1. Map of Belgium, with the five study sites (black dots) located in the main ecological regions of Belgium.

yet permanently present in pasture by day or by night. Farmers have not yet sprayed pesticides on crops, and *Culicoides* emergence and reproduction has just begun. Autumn is the end of the grazing season. Cattle are still present during the day in pastures but the duration of grazing is shortened. Insecticides have been spread on crops on several occasions. The spring sampling was carried out between April and early May 2009, and the autumnal sampling was carried out in October 2008. Meteorological conditions during sampling were recorded with a portable weather station (Ventage Pro 2; Davis, Hayward, CA, U.S.A.), set in the pasture.

Experimental design

Eight standard Onderstepoort-type black-light traps (Venter *et al.*, 1996; Goffredo & Meiswinkel, 2004) with new UV-light tubes (F8T5 BLB) were independently supplied with eight 12-V calcium batteries (44Ah, 210A) and DC/AC power inverters (100 Watt). They were set at a height of 1.80–2.2 m, using the shelter-building structure for the first trap of the transect, poles inserted in the ground in the pastures and branches of trees in woodlands. Traps were located at increasing distances from the farm as illustrated in Fig. 2. The first trap (i) was located outside the main cattle shelter to ensure comparability of the method to standard sampling protocols (Goffredo & Meiswinkel, 2004). The second trap (ii) was located at the nearest point of the pasture from the shelter. The third to sixth traps (iii–vi) were placed at increasing distances of 20, 50, 100 and 200 m from the second trap (ii). Finally the seventh (vii) and eighth traps (viii) were, respectively, set at the edge of the forest and 100 m inside the forest in spring and 200 m in autumn. The Onderstepoort-type black-light traps were randomized at each sampling date.

The peak in *Culicoides* spp. activity is reported to occur just after sunset (Service, 1971; Edwards *et al.*, 1987; Anderson & Linhares, 1989; Blackwell, 1997; Kettle *et al.*, 1998; Gerry

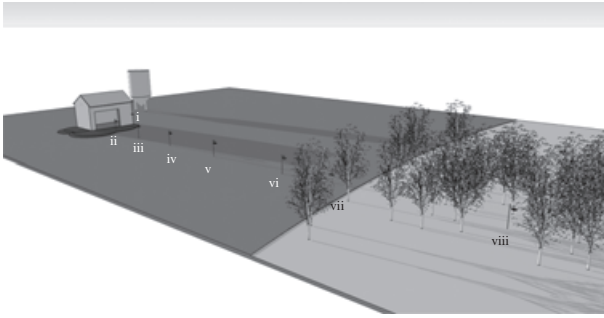


Fig. 2. Schematic view of the transects from the main shelter with hosts (trap in position i), within pastures (ii to vi), at the edge of forests (vii) and inside forests (viii). The distances presented in this view are not scaled.

et al., 2009; Rigot & Gilbert, 2011). Sampling was hence conducted from 1 h before the end of civil twilight (ECT) until 2 h afterwards in the autumn and from 30 min before ECT until 1 h 30 min afterwards in spring. There were no large mammals near the light traps set in pastures at the time of sampling. Cattle were present in the farm, and wild mammals may have been present in the forest without being noticed. As a result of logistic constraints, traps were turned on manually with 5-min intervals between each. However, the turning-on sequence was the same at all sites for each replicate: from inside the animal shelter were always collected 35 min after the initial sampling carried out in the forests, although the total duration of the sampling was constant throughout the transect. The experiment was repeated during two consecutive nights for each of the five sites in each of the two seasons.

Sample identification

Insects were stored in absolute ethyl alcohol (AnalaR Normapur; VWR Int., Leuven, Belgium) and brought to the laboratory to be identified. Collections larger than 5000 insects were sub-sampled according to the method described by Van Ark & Meiswinkel (1992) and Goffredo & Meiswinkel (2004). Females and males belonging to the genus *Culicoides* were differentiated from remaining insects (mainly psychodids) based on their wing patterns and general shape (Delécolle, 1985; Goffredo & Meiswinkel, 2004). Females were identified to the species level according to the numerical key of Mathieu *et al.* (2010) and when possible, separated into nulliparous or parous groups according to their abdominal pigmentation (Dyce, 1969). Gravid and blood-fed females were pooled with parous ones. Undifferentiated females were pooled in an undetermined group.

Simulations

The present study aimed to verify how the pattern of catches as a function of distance to the farm could be influenced by the experimental design, and more specifically by possible

competition between traps. Simulations detailed in Appendix S1 were therefore carried out in order to characterize the spatial patterns that would have been obtained if randomly distributed insects had been caught by traps competing with each other.

Quantitative analyses

The distribution of catches as a function of the distance to the farm was studied according to the works of Bateman (1950), Wallace (1966) and Kot *et al.* (1996). Three models were tested to fit the experimental data:

1. Linear: $N \sim \alpha + \beta d$
2. Gaussian: $N \sim \alpha e^{(-d^2/\beta)}$
3. Leptokurtic: $N \sim \alpha e^{(-\beta d)}$,

where N = number of individuals and d = distance to the farm

The parameters of the linear model were estimated using a generalized linear model (GLM, Venables & Ripley, 2002), while the parameters of the Gaussian and Leptokurtic models were estimated by maximum likelihood (MLE, Bolker, 2008). For all models, we assumed a negative binomial distribution to account for the overdispersion in the response variable and a log-link function. Data were not log-transformed (O'Hara & Kotze, 2010). Akaike's information criterion (AIC) and root mean square error (RMSE) were used to select the best model. The influences of season and species on model parameters were investigated by comparing different nested models with likelihood ratio tests (LRT) (Bolker, 2008).

Finally, the current study also tested if the abundance and composition of *Culicoides* was related to the three types of land use covered by the transects: farms, pastures and forests. Samples collected at the level of farms were pooled in the first type. The second type included samples collected in the pastures at a distance > 100 m from the farm, in accordance with the distance of independence between Onderstepoort light trap catches established in Rigot & Gilbert (2011). The third and fourth types included samples caught at the forest edge and inner forests, respectively. The effects of season, parous vs. nulliparous females and species were also tested in the analyses. These comparisons were achieved using analysis of deviances on negative binomial GLM fits and chi-squared tests (Hastie & Pregibon, 1992). As the high number of zeros encountered for some categories of species, data were filtered to include only collections with abundances of a given species higher than a threshold value. This value was arbitrarily set to 5% of the mean abundances of the studied species. Both types of results, with or without a filter, are presented only when they met the requirement of homoscedasticity. All analyses were carried out using R (R Development Core Team, 2010).

Results

Overall, 39 153 insects were caught during the 20 nights of sampling, corresponding to a total of 160 light trap samplings

Table 1. Total numbers of female *Culicoides* collected during the survey.

Female <i>Culicoides</i>	Spring			Autumn		
	Nulliparous	Parous	Undetermined	Nulliparous	Parous	Undetermined
<i>Avaritia</i>		13 298			777	
<i>C. obsoletus/C. scoticus</i>	46%	30%	≤0.5%	36%	18%	19%
<i>C. chiopterus</i>	≤0.5%	22%	≤0.5%	1%	16%	≤0.5%
<i>C. dewulfi</i>	1%	1%	≤0.5%	3%	6%	≤0.5%
<i>Culicoides</i>		1797			101	
<i>C. pulicaris/C. lupicaris</i>	26%	19%	2%	32%	30%	26%
<i>C. punctatus</i>	8%	34%	4%	7%	5%	—
<i>C. newsteadi</i>	—	1%	—	—	—	—
Impunctatus group*	1%	4%	≤0.5%	—	1%	—
<i>Other subgenera</i>		369			5	
		Undistinguished			Undistinguished	
<i>C. pictipennis</i>		66%			—	
<i>C. brunicans</i>		11%			—	
<i>C. vexans</i>		12%			—	
<i>C. nubeculosus/C. puncticollis</i>		6%			—	
<i>C. riouxi</i>		2%			—	
<i>C. salinarius/C. circumscriptus</i>		≤1%			—	
<i>C. heliophilus</i>		≤1%			2	
<i>C. stigma/C. parroti</i>		—			2	
<i>C. minutissimus</i>		—			1	
Other species		≤1%			—	

**C. impunctatus*, *C. fagineus*, *C. deltus* and *C. grisescens*.

(112 in Spring and 48 in Autumn). Among these, 41.8% (16 347) and 1.4% (555) were identified as females and males of *Culicoides* spp. Female *Culicoides* were mainly represented by specimens belonging to *Avaritia* and *Culicoides* subgenera (Table 1). Among these females, 97% belonged to five species or groups of species: *Obsoletus* complex (*C. obsoletus* Meigen / *C. scoticus* Downes & Kettle), *C. chiopterus* Meigen, *C. dewulfi* Goetghebuer, *Pulicaris* group (*C. pulicaris* Linnaeus / *C. lupicaris* Downes & Kettle) and *C. punctatus* Meigen. Parous and nulliparous females were collected in comparable abundance ($\theta = 0.2$, deviance = 0.3, d.f. = 1,314, $P = 0.56$). The remaining 56.8% of insects (22 251) belonged to the family Psychodidae (78.2%– 17 405, 44.5% of the total).

Pooled data on female or male *Culicoides* collected at the level of each trap are presented in Fig. 3A and B. Overall, there was a general decrease in the number of female *Culicoides* spp. collected in the pastures with increasing distance from the farms. Their number appeared to increase when the transects entered the forests (trap positions vii and viii). Males were always caught in much lower numbers than females. In autumn, they were only detected near the farms. In spring, their distribution appeared to be homogeneous. Random distribution simulations did not match the patterns of distribution observed in experimental data (Fig. S1).

The effect of distance on the total number of female *Culicoides* collected from up to 250 m away from the farms was significant in all the models tested (Table 2). Akaike's information criterion values were similar for the Linear and Leptokurtic models and lower than that found for the Gaussian model (dAIC = 5.4). The RMSE was significantly lower for the Leptokurtic model than for the Linear or Gaussian models (ANOVA $F = 528.53$, d.f. = 2,297, $P < 0.001$, Tukey $\alpha = 95\%$, $P < 0.001$). The Leptokurtic model was hence selected and is presented in Fig. 4. Accounting for the difference between seasons in the model significantly reduced the AIC (LRT $\chi^2 = 21.1$, d.f. = 2, $P < 0.001$, Fig. 4, Table 3). In addition, breaking down the model by species significantly improved the model fit (LRT $\chi^2 = 130.3$, d.f. = 10, $P < 0.001$). When breaking down the analysis species by species, a significantly decreasing pattern of catches modelled by the leptokurtic function was found for *Obsoletus* complex, *C. chiopterus*, *Pulicaris* group and *C. punctatus*. For male *Culicoides*, no significant association was found between the number of catches and distance from the farms. In autumn, they were caught only on farms. In spring, their distribution as a function of the distance from the farms was best described by a linear relation with a slope not differing significantly from 0 ($\alpha \pm \text{SE} = 3.39 \pm 0.95$, $P < 0.001$). Because of the difference between autumn and spring catches, accounting for the season in the model of male

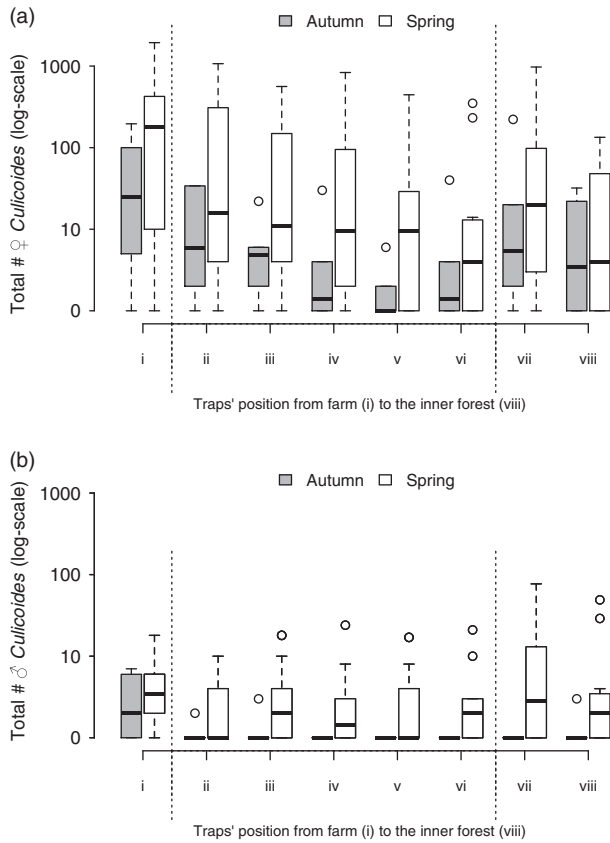


Fig. 3. Abundances of female (a) and male (b) *Culicoides* at increasing distances from farms (i), in pastures (ii to vi), and further apart, at the edge (vii) and inside (viii) woodlands; in spring (white box-and-whisker plots) and autumn (grey box-and-whisker plots). Open circles represent potential outlier data points outreaching by more than 1.5 times the inter-quartile range. Dashed lines were drawn to delineate farm and forest habitats (respectively on the left and on the right) from pastures.

catches resulted in a significant improvement of the fit (LRT $\chi^2 = 30.93$, d.f. = 2, $P < 0.001$).

Total abundances of female *Culicoides* collected across different land use types (farms, pastures, forest edges and inner forests) are shown in Fig. 5A. The effect of land use types on female catches was significant, as was season (Table 4). Furthermore, for each season considered separately, the differences observed between each type of land use are

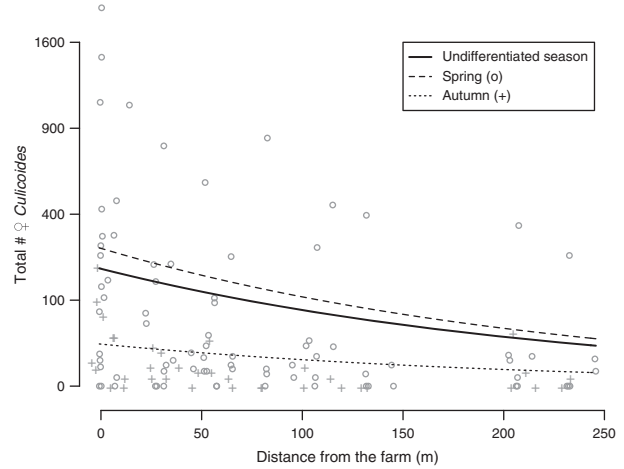


Fig. 4. Leptokurtic models of total female *Culicoides* caught as a function of the distance from the farm: in autumn (dotted line), in spring (dashed line) and without distinction between seasons (solid line). See parameters in Tables 2 and 3. A random jitter (± 10 m) has been added to the distance so that overlapping points can be visualized. For visual convenience, data and models were square-root scaled.

summarized in Table 5. In the autumn, catches in pastures were significantly lower than in all other land use types. In spring, very few differences appeared to be significant except that farms had significantly more catches than pastures (farms > pastures, $P = 0.01$). In both seasons, farms and forest edges had comparable mean catches. In spring, lower abundances of catches were found in the inner forests, with levels comparable to pastures.

The effect of species was found to be significant when added to the model, together with land use type and season (Table 6). The interactions that may be present between the four groups of land use and species abundances are illustrated in Fig. 5B and C for each season considered separately. The general patterns shown by the individual species are similar to the one showed by the pooled female *Culicoides*, i.e. higher catches in farms, reduced catches in pastures, catches increasing when the traps are located in the forests near the edge and lower in the inner forests than at the edges (e.g. *Obsoletus c.* and *C. chiopterus*). By contrast, particularly in spring, some species groups showed a pattern of distribution that was unaffected when the transect entered the forests (e.g. Pulicaris group and *C. dewulfi*). The significant differences observed between

Table 2. Comparison of different models of *Culicoides* catch reduction as a function of the distance to the farm.

Models	Parameters estimation	\pm SE	θ^*	AIC	RMSE	
Linear	α	5.23 \pm 0.30	***	0.20 \pm 0.03	1055.2	309.7
	β	-0.009 \pm 0.003	**	—	—	—
Gaussian	α	209.83 \pm 63.09	***	0.19 \pm 0.02	1060.6	289.1
	β	20 077.6 \pm 3607.9	***	—	—	—
Leptokurtic	α	186.41 \pm 52.37	***	0.20 \pm 0.03	1055.2	281.9
	β	0.009 \pm 0.003	***	—	—	—

*Negative binomial dispersion parameter ($P \leq 0.001$).

Table 3. Parameters of the leptokurtic models of *Culicoides* catches as a function of distance from the farm, and the effect of season and species.

Leptokurtic models	Parameters estimation	95% CI	-2log L
Seasonal effect	θ 0.24***	0.18; 0.30	1028.03
Autumn	α 23.90*	10.90; 66.29	
	β 0.0093*	0.001; 0.017	
Spring	α 256.90**	147.58; 511.75	
	β 0.0087**	0.018; 0.30	
Species effect			
1/grouped signal†	θ 0.09***	0.08; 0.11	3116.25
	α 30.74*	22.59; 44.23	
	β 0.0085*	0.0056; 0.012	
2/species-specific signals	θ 0.13***	0.11; 0.14	2985.98
Obsoletus group	α 114.00**	57.56; 251.96	
	β 0.0073*	0.0020; 0.0114	
<i>C. chiopterus</i>	α 49.00**	25.21; 107.05	
	β 0.018*	0.010; 0.023	
<i>C. dewulfi</i>	α 2.90**	1.49; 6.30	
	β 0.0050 NS	-0.0015; 0.0109	
Pulicaris group	α 9.63**	5.15; 20.39	
	β 0.0079*	0.0018; 0.0128	
<i>C. punctatus</i>	α 11.00**	5.40; 26.58	
	β 0.011**	0.005; 0.019	
Remaining specimens	α 2.74**	1.42; 6.16	
	β 0.0033 NS	-0.0038; 0.0096	

†Model obtained by ML fit on *C. dewulfi*, *Pulicaris* group, *C. punctatus* and remaining specimens ungrouped.

species abundances within each type of land use are presented as Table S1.

Discussion

The study provided two main results on the distribution of Palearctic *Culicoides* catches at fine spatial scales: (a) a pattern of decreasing abundance as a function of the distance from

Table 4. Effects of land use and season on the total number of female *Culicoides* caught in the samples.

	df	Deviance	Resid. df	Resid. dev.	P(> Chi)
NULL	—	—	88	136.41	—
Landuse	3	17.51	85	118.90	<0.001
Landuse:season	4	18.44	81	100.47	= 0.001

$\theta(\pm \text{SE}) = 0.24 (\pm 0.04)$; $-2\log L = 755.51$.

Terms of nested models have been included sequentially in the deviance table (first to last): 'Null' indicates the initial model that does not differentiate land use. 'Land use' is the model accounting for land use types, and 'Land use: season' refers to the model accounting for seasonal variations within land use types.

farms and which varied according to season, species and sex; and (b) a higher abundance of female *Culicoides* in the forests than expected considering the distance of forest sampling sites from farms, and this varied according to species.

The abundance of female *Culicoides* decreased as distance from farms increased. This was best quantified by a leptokurtic trend, a pattern very common in animals and plants (e.g. Kot *et al.*, 1996). This pattern may simply reflect emerging individuals flying at random from their breeding sites on farms (Kettle, 1951; Zimmerman & Turner, 1983). However, farms may not be as rich in roosts as previously suggested (Zimmer *et al.*, 2010), and the observed pattern may also result from a high attraction by the hosts, as commonly observed in mosquitoes (Gillies & Wilkes, 1970) and observed for *Culicoides* midges by Kettle (1960) in Zimmerman & Turner (1984), Jones & Akey (1977), Koch & Axtell (1979), Schmidtman *et al.* (1980, 1981), Zimmerman & Turner (1983) and Garcia-Saenz *et al.* (2010).

The decrease in female number from farms to pastures was less marked in spring than in autumn (Figs 3, 4 and 5). This pattern may depend on the difference in microclimatic conditions between the pasture open habitat, and the more closed habitat formed by farms or forests (e.g. temperature and wind). *Culicoides* flight activity might be higher near the farms than in the pastures, especially when meteorological conditions for flight are poor, such as in the autumn. In addition, as a result of farming practices and the reduction in day length, the presence of cattle near or inside shelters

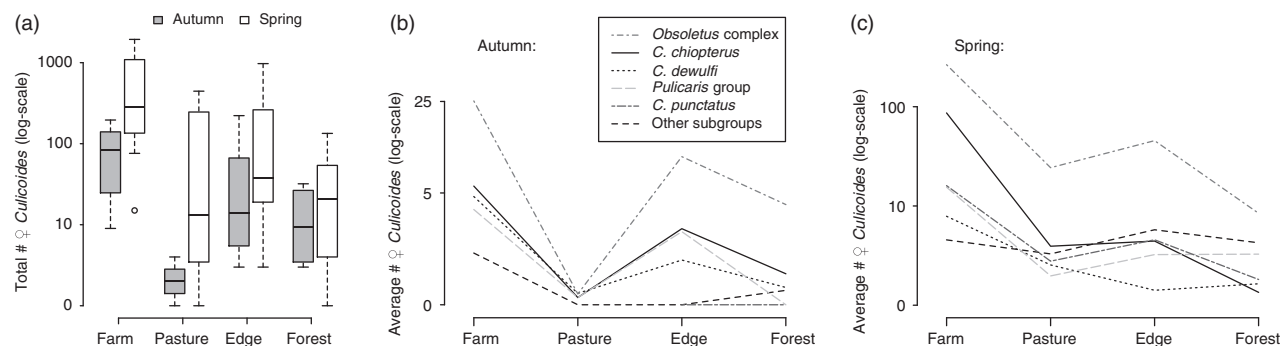
**Fig. 5.** Distribution patterns of female *Culicoides* in four types of land use, in the autumn (dark grey box-and-whisker plots) and in the spring (white box-and-whisker plots) (a) and species (b and c). All data presented here are filtered (see 5% filter presented in method) and log-transformed.

Table 5. Comparisons between the abundances of total female *Culicoides* in different land use types, in autumn and spring.

	Farm	Pasture	Edge forest	Forest	
Farm		> **/**	NS/> ^o	> ***/**	Spring
Pasture	< ***/**		NS/NS	NS/<	
Edge forest	NS/NS	> ***/**		> /**	
Forest	< /**	> ***/**	NS/<		
		Autumn			

'<' and '>' refer to smaller or higher, respectively, abundances in the land use type specified in the columns and row. Levels of significance selected are 0.1 (^o), 0.05 (*), 0.01 (**), and 0.001 (***). Results based on the filtered data (see 5% filter in methods) are shown in gray.

Table 6. Effects of land use and season on the total number of female *Culicoides*, accounting for the effect of species.

	df	Deviance	Resid. df	Resid. dev.	P(> Chi)
NULL	—	—	533	626.09	—
Species	5	109.61	528	516.48	<0.001
Species:landuse	18	73.48	510	443.00	<0.001
Species:landuse:season	24	77.71	486	365.29	<0.001

$\theta(\pm SE) = 0.14 (\pm 0.01)$; $-2\log L = 2067.54$.

Terms of nested models have been included sequentially in the deviance table: 'Null' refers to the initial model that does not account for different species, 'Species' refers to the model accounting for the effect of species, 'Species:land use' refers to the model accounting for different land use types within different species and 'Species: land use:season' refers to a nested model accounting for an effect of the season within different land use types and for different species.

is higher in the autumn than in the spring. Indeed, autumn is the season of the year when animals tend to aggregate near resting areas at sunset rather than grazing further apart in the pastures (Gonyou & Stricklin, 1984). One should note that the unfavourable meteorological conditions during our autumn sampling in comparison to the spring sampling (average temperature was 8.8 °C vs. 11.9 °C and wind speed = 0.42 m/s vs. 0.27 m/s) could also explain the difference in relative abundance highlighted in Fig. 4. This difference could also be partly caused by chemical treatments applied around farms at different times of the year, and future studies should aim to assess this possible effect.

Most species and species groups showed significantly lower catches with increasing distance from the farms, indicating that the pattern identified in the pooled samples reflects a trend at the genus level and does not result from antagonist species-specific signals. However, some of the model parameters differed between species or species-groups (Table 3). For example, *C. chiopterus* females, the second most abundant species collected at the level of the farms, showed the strongest drop in catches as a function of distance from the farm (Table 3 and Figure S2). A strong or weak decrease in catches when distance from the farm increases could result from species-specific

behaviours. Future behaviourally-oriented studies should aim to better understand the biological cause of these species-specific responses.

Male *Culicoides* tended to be homogeneously distributed from the farm to pasture in spring. In the autumn, they were only detected at the level of the farm. This suggests that the pattern observed in females may reflect their attraction towards their hosts rather than their random dispersal away from farm breeding sites. Indeed, although little is known about female and male flight behaviour after emergence, if the trend in females was caused by breeding sites concentrated in the farm, one would have expected a similar pattern in males. In addition, the number of males caught was relatively low which could result from various factors such as an unequal mortality of sexes at emergence, or alternatively, a difference in the response to the trap stimulus. We are hence somewhat limited in our interpretation of this result.

An important and somewhat unexpected result is the increase in the numbers of *Culicoides* collected when the transect entered the forest, at a much higher level than one would have expected based on the distance of the traps from the farm. This result strongly suggests that farms are not the only element structuring BTV vector populations at the landscape scale. Previous works have already suggested the presence of *Culicoides* in northern-European forests (Kettle, 1951; Service, 1971; Meiswinkel *et al.*, 2008) and highlighted the potential contribution of natural areas in the maintenance and spread of BTV, especially as most of the species encountered in these studies have been recognized as BTV vectors (e.g. Carpenter *et al.*, 2006; Mehlhorn *et al.*, 2007; Dijkstra *et al.*, 2008). However woodlands, and more generally natural areas, are strongly underrepresented in large-scale surveillance entomological surveys. With midges being found in abundance at the edge of and within the forest, the possible transmission of BTV to and from wild ungulate populations is likely and should be further explored. This is further supported by the recent works of Bartsch *et al.* (2009), Lassen *et al.* (2010) or Ninio *et al.* (2010) who showed that *Culicoides* collected near farms may contain blood from wild hosts, and the study of Linden *et al.* (2010), showing BTV-8 infection in northern-European wild hosts.

The relatively high *Culicoides* abundance in woodlands was not identical for all species and one may expect it to be low for species associated to livestock. This study confirmed this hypothesis with low catches in woodland for *C. dewulfi*, which is known to mainly breed in livestock dung (e.g. Zimmer *et al.*, 2008). For other species, such as those of the Pulicaris group, forest may provide a high diversity of roosts (Blackwell *et al.*, 1999), and possibly also wildlife hosts.

In conclusion, results of the present study indicate that farms and forests are two key elements structuring the population of Palearctic *Culicoides* at the landscape scale, and may hence influence the transmission of BTV to domestic hosts. More specifically, the role of natural and semi-natural woodlands would need deeper investigation so as to better characterize their potential role as BTV risk factors (Szaragd *et al.*, 2009, 2010).

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: DOI: 10.1111/j.1365-2915.2012.01032.x

Figure S1. Simulations' patterns for low (black line) and high (grey line) particles' densities when attraction radii are equal to 25 m (a) or higher (50 m, b).

Figure S2. Species-specific leptokurtic models of female *Culicoides* abundances as a function of the distance to the farm. Coefficients used herein were presented in Table 3.

Table S1. Significant comparisons resulting from the negative binomial generalized linear model (GLM) fits (described in Materials and Methods) for each season investigated separately: (a) autumn and (b) spring. The grey colour shows results of statistical analyses on filtered data (see 5% filter in methods). '>' means 'is higher than'; '<' means 'is lower than'; '*', '**' and '***' stands respectively for levels of significance fixed to 0.05, 0.01 and 0.001. Levels comprised between 0.05 and 0.1 are also presented.

Appendix S1. Simulation runs: the method to account for spatial randomness and light traps interferences among samples.

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