

Factors affecting *Culicoides* species composition and abundance in avian nests

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SUMMARY

Mechanisms affecting patterns of vector distribution among host individuals may influence the population and evolutionary dynamics of vectors, hosts and the parasites transmitted. We studied the role of different factors affecting the species composition and abundance of *Culicoides* found in nests of the blue tit (*Cyanistes caeruleus*). We identified 1531 females and 2 males of 7 different *Culicoides* species in nests, with *C. simulator* being the most abundant species, followed by *C. kibunensis*, *C. festivipennis*, *C. segnis*, *C. truncorum*, *C. pictipennis* and *C. circumscriptus*. We conducted a medication × fumigation experiment randomly assigning bird's nests to different treatments, thereby generating groups of medicated and control pairs breeding in fumigated and control nests. Medicated pairs were injected with the anti-malarial drug Primaquine diluted in saline solution while control pairs were injected with saline solution. The fumigation treatment was carried out using insecticide solution or water for fumigated and control nests respectively. Brood size was the main factor associated with the abundance of biting midges probably because more nestlings may produce higher quantities of vector attractants. In addition, birds medicated against haemoparasites breeding in non-fumigated nests supported a higher abundance of *C. festivipennis* than the rest of the groups. Also, we found that the fumigation treatment reduced the abundance of engorged *Culicoides* in both medicated and control nests, thus indicating a reduction of feeding success produced by the insecticide. These results represent the first evidence for the role of different factors in affecting the *Culicoides* infracommunity in wild avian nests.

Key words: blue tit, *Culicoides* spp., Primaquine, avian nests.

INTRODUCTION

The study of biting midges of the genus *Culicoides* Latreille (Diptera: Ceratopogonidae) is of great importance not only because females are obligate blood feeders attacking a huge diversity of vertebrates (Downes, 1958; Kettle, 1995; Marquardt *et al.* 2000), but also because they are vectors of a large number of transmissible agents. Some of these pathogens, including viruses (Braverman *et al.* 1996; Mellor *et al.* 2000) and other parasites such as protozoa and filarial worms (Fallis and Wood, 1957; Atkinson *et al.* 1983; Shelley and Coscarón, 2001; Garvin and Greiner, 2003; Mullens *et al.* 2006), have economic and veterinary importance.

Females of biting midges, the only sex that requires blood, are infected by blood parasites when they obtain a meal from an infected host. With the exceptions of few non-biting species and autogenous species that require a bloodmeal only after laying their first egg batch, most *Culicoides* females need to obtain blood for their first ovarian development (Downes, 1958). Many studies on *Culicoides* have been conducted to identify the mechanisms affecting their host selection processes and feeding patterns. However, in the wild, there is scant information about ecological relationships between *Culicoides* and their hosts, especially in the case of wild birds. The main reason for the scarcity of this kind of study is probably the absence of an effective method of capture. Usually, biting midges are captured using different gadgets such as light traps, CO₂ traps placed close to the animals or directly vacuuming them from the animals' bodies (i.e. Bennett, 1960; Braverman *et al.* 1976; Zimmerman and Turner, 1983; Mushi *et al.* 1999; Yu *et al.* 2000; Mullens

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et al. 2005). However, these methods are difficult to use in avian nests, especially for the study of midges attacking birds of species breeding in nests placed in cavities.

Arthropod–host interactions involve fascinating behavioural processes and chemosensory mechanisms and chemicals that allow vectors to express host-selection behaviours resulting in non-random biting (Mukabana *et al.* 2002; Tomás *et al.* 2008*b*). Visual as well as antennal and maxillary receptors may be involved in host-location (Bowen, 1991). *Culicoides* have receptors sensitive to a diversity of host derived products such as lactic acid, 1-octen-3-ol and CO₂ (Bhasin *et al.* 2000*a*; Grant and Kline, 2003), which produce attractive effects (Blackwell *et al.* 1996; Gibson and Torr, 1999; Marquardt *et al.* 2000; Mordue, 2003; Mands *et al.* 2004). Also, the presence of volatile pheromones produced by parous midge females may attract other females, as reported by Blackwell *et al.* (1994) in their study of an autogenous species, the biting midge *C. impunctatus*. In addition, as may occur under natural conditions, host-derived volatile components may interact with parous female pheromones, either attracting or repelling females as a function of the relative doses of each chemical (Blackwell *et al.* 1996).

Also, host infection status may be a key factor affecting host location by vectors, because infection could affect host metabolism and therefore host-derived attractants (Torres-Estrada and Rodríguez, 2003; Lacroix *et al.* 2005). In humans, individuals with high intensities of infection by malaria are more susceptible to the attack by vectors (Lacroix *et al.* 2005). However, this may not be the case for birds (Tomás *et al.* 2008*b*) where higher abundances of biting midges were found in nests of female blue tits with experimentally reduced intensities of infection by medication with an anti-malarial drug, an effective method to reduce the intensity of infection by the *Culicoides* transmitted malaria-like *Haemoproteus* (Merino *et al.* 2000; Tomás *et al.* 2005; Martínez-de la Puente *et al.* 2007). Biting midges may prefer to feed on less infected birds because blood parasites may reduce their survival (Valkiūnas and Iezhova, 2004). On the other hand, the infection status could also affect host susceptibility to vector attacks through other ways such as reducing host antimosquito behaviours (Torres-Estrada and Rodríguez, 2003). It is known that hosts use a diversity of insect-repelling strategies to avoid the attack of biting midges including anti-insect behaviours (Edman *et al.* 1974; Mooring *et al.* 2003; Darbro and Harrington, 2007) or the use of plants with insecticide properties (Bucher, 1988; Clark, 1991; Lafuma *et al.* 2001). Humans, due to the sanitary and economical importance of *Culicoides* (Mellor *et al.* 2000; Ratnayake *et al.* 2006), also use different insecticides to control midge populations. There is evidence of lower abundances of *Culicoides*

in fumigated farms as compared to non-fumigated ones (Sarto i Monteys and Saiz-Ardanaz, 2003; also see Satta *et al.* 2004) that may reduce the costs associated with the activity of biting insects. In the case of birds, some species introduce in their nests plants with insect-repellent properties that could reduce the abundance of ectoparasites in avian nests (Bucher, 1988; Clark, 1991). Both naturally derived and synthesized components have been tested for their repellent effect on biting midges (Braverman and Chizov-Ginzburg, 1997). In wild populations, birds may also benefit from the use of insecticides if they reduce biting midge densities. In the case of blue tits *Cyanistes caeruleus*, it has been suggested that the use of green plants could be a mechanism of protection against parasites (Cowie and Hinsley, 1988; Banbura *et al.* 1994; Petit *et al.* 2002). However, the effect of plant-derived repellents could be different among parasite species because there are both attraction and repellency effects of a particular compound among *Culicoides* species (Braverman *et al.* 1999). To reveal the potential effect of insecticides on *Culicoides* infracommunities in avian nests, studies in wild populations should be performed. In this respect, we found in a previous study that the use of an insecticide treatment was not effective in reducing the abundance of *Culicoides* in blue tit nests, although a differential specific susceptibility of *Culicoides* species to the insecticide treatment could affect these results (Tomás *et al.* 2008*a*).

Understanding the interactions between biting midges and birds is especially interesting for the case of hole-nesting species because some of these birds predate on insect pests of gardens and forests. Also it is important to note that *Culicoides* is a worldwide distributed genus, with about 1254 described species (Beckenbach and Borkent, 2003), present in most terrestrial habitats (Kettle, 1995; Marquardt *et al.* 2000). Our aim in this study was to identify the role of different factors affecting the composition and abundance of *Culicoides* species in a wild population of a hole-nesting bird, the blue tit. In addition, we investigated the abundance of parous and engorged *Culicoides* females, because parous females are potential haemoparasite vectors and engorged females have fed recently on a host. In addition, we investigated the abundance of nulliparous females because, although they have not fed, they are potential haemoparasite vectors to the same degree as life stages after feeding.

MATERIALS AND METHODS

Study area

This study was carried out in a population of blue tits *Cyanistes caeruleus* breeding in nest-boxes during the spring of 2005 in a Pyrenean Oak *Quercus pyrenaica*

deciduous forest located in Valsaín, Central Spain (Segovia, 40°53'74N, 4°01'W, 1200 m a.s.l.).

Treatments

When nestlings were 3 days old, nests were randomly assigned to fumigation and medication treatments, thereby generating groups of medicated and control pairs breeding in fumigated and control nests (14 medicated × fumigated nests, 14 medication control × fumigated nests, 15 medicated × fumigation control nests and 16 medication control × fumigation control nests). The medication consisted in a subcutaneous injection of 0.1 ml of the anti-malarial drug Primaquine (Sigma, St Louis, MO, USA) diluted in saline solution (concentration 1 mg·ml⁻¹) when nestlings were 3 days old. Control pairs were injected with the same volume of saline solution. Treatment with Primaquine causes a reduction in the intensity of infection by blood parasites in the study population (Merino *et al.* 2000; Tomás *et al.* 2005; Martínez-de la Puente *et al.* 2007). The fumigation treatment was carried out at 3 different times (at the nestling ages of 3, 7 and 11 days) with an insecticide solution (Stockade©, Fort Dodge Veterinaria, S.A., Vall de Bianya, Girona, Spain) comprising 0.5% Permethrin and 1% Piperonyl butoxide. Nestlings were extracted from nests prior to fumigation and left again in the nest immediately after treatment. This treatment has been previously used to reduce ectoparasite populations in nests without detection of any deleterious effect for nestlings (Tomás *et al.* 2007b). The same methodology was employed in control nests using water instead of insecticide.

Culicoides collection and identification

During 2 days after the last fumigation, *Culicoides* were captured using the method described and tested by Tomás *et al.* (2008a). This method consisted in the placement inside the nest-boxes of plastic Petri dishes (8.5 cm diameter; 56.7 cm²) layered with 0.5 ml of commercially available body gel-oil (Johnson's© baby chamomilla, Johnson and Johnson, Dusseldorf, Germany). This gel-oil is made up of paraffinum liquidum, hexyl laurate, ethylene/propylene/styrene copolymer, cyclopentasiloxane, butylene/ethylene/styrene copolymer, chamomilla recutita, bisabolol and perfume [FPT1353]. The effect of the fumigation treatment in non-medicated pairs was previously reported by Tomás *et al.* (2008a) in the context of a methodological study to determine the efficacy of such a sticky medium to collect biting midges. On day 13, brood sizes for each nest were recorded and Petri dishes removed and stored in a freezer until their examination.

In the laboratory, biting midges were removed from dishes using xylene and maintained in absolute ethanol until their identification. All *Culicoides* species were initially sorted depending on

their wing pattern under an Olympus SZH stereo-microscope (10×–64× magnification). However, given their minute size (usually no longer than 3 mm), for more accurate diagnosis, it was necessary to dissect many of the midges and make microscopic slide preparations of their body parts. For fixing them we used Tendeiro solution (distilled water: 35 ml; chloral hydrate: 40 g; glacial acetic acid: 18 ml; polyvinyl alcohol: 7 g). To identify them to specific level we used Kremer's (1966) and Delécolle's (1985) morphological keys. *Culicoides* were sexed and the parity of females determined as follows: nulliparous (those that have never fed on blood), parous (those showing a burgundy pigment in the subcutaneous cells of the abdomen indicating a previously digested bloodmeal; see Dyce (1969) or engorged females (those with a bloodmeal still not completely digested in their abdomen). We assume that engorged females fed on blood from birds (nestlings or adults) from the nest-box where they were captured.

Statistical analysis

Total abundance of *Culicoides* and each specific abundance were logarithmically (log₁₀) transformed to normalize distributions. General regression models (GRM) (Statistica version 6.0, StatSoft, Inc. 2001) applying the forward stepwise solution, were used to investigate the relationships between the total abundance of *Culicoides*, the abundance of total nulliparous *Culicoides* females and the abundance of each species, including in the model the 2 treatments (fumigation and medication treatments) and their interaction as factors and brood size and phenology (a potential confounding variable estimated as hatching date of each brood) as covariables. Results were also confirmed using backward stepwise solutions. Residuals of the models were tested for normality. Variables reflecting total abundances included the total number of nulliparous, parous and engorged females per nest. In addition, when residuals of the models did not follow a normal distribution, non-parametric analyses were conducted. Simple correlations and Mann-Whitney U-tests were used to test for the effect of each brood size, seasonality and fumigation and medication treatments on the species richness, the abundance of total parous females and the abundance of total engorged females (both not normally distributed variables, even after log transformation). Analyses for *Culicoides* species were restricted to the 3 more abundant species, *C. simulator*, *C. kibunensis* and *C. festivipennis* (see Table 1).

RESULTS

A total of 1531 female biting midges of 7 different species were captured in 57 nests. Only 2 males

Table 1. Abundance of each female stage (nulliparous, parous and engorged) for each *Culicoides* species captured in blue tits nests during the breeding season of 2005

(The percentage of infected nests is shown in parentheses. As several stages were present in the same nests the sum of percentages is higher than 100. However the column total shows the percentage of nests infected by each species.)

Species	Nulliparous	Parous	Engorged	Total
<i>C. simulator</i>	751 (86.4)	76 (35.6)	44 (45.8)	871 (93.2)
<i>C. kibunensis</i>	322 (79.7)	42 (37.3)	9 (11.9)	373 (81.4)
<i>C. festivipennis</i>	121 (61.0)	60 (30.5)	5 (5.1)	186 (62.7)
<i>C. segnis</i>	59 (42.4)	9 (13.6)	7 (11.9)	75 (50.8)
<i>C. truncorum</i>	14 (13.6)	3 (5.1)	2 (3.4)	19 (20.3)
<i>C. pictipennis</i>	2 (3.4)	2 (3.4)	0 (0)	4 (6.8)
<i>C. circumscriptus</i>	3 (5.1)	0 (0)	0 (0)	3 (5.1)
Total	1272 (91.5)	192 (62.7)	67 (55.9)	1531

Table 2. GRM results after applying the forward stepwise solution for the relationship between the total abundance of *Culicoides* and the abundance of each species with the medication and fumigation treatments and their interaction, brood size and phenology

(Adjusted R² values are shown.)

Dependent variable	Model	Retained variable		
Total abundance	R ² =0.11	P<0.01	Brood size	F _{1,57} =7.83 P<0.01
<i>C. simulator</i>	R ² =0.13	P<0.003	Brood size	F _{1,57} =9.72 P<0.003
<i>C. kibunensis</i>	R ² =0.18	P<0.002	Brood size	F _{1,56} =12.37 P<0.001
			Phenology	F _{1,56} =8.19 P<0.01
<i>C. festivipennis</i>	R ² =0.06	P=0.03	Medication * Fumigation interaction	F _{1,57} =4.73 P=0.03

(one *C. kibunensis* and one *C. festivipennis*) were captured. In 2 additional nests we did not capture any biting midge (Table 1). In addition, 41 biting midges (2.6% of the total) could not be identified because of the absence of wings or other anatomical structures. However, unidentified individuals were also considered in total abundances. In each nest, we captured an average of 26.6 (s.d. 39.1, range 0–208) biting midges from 3.1 (s.d. 1.5, range 0–6) different species.

The abundance of total *Culicoides* females captured in avian nests was strongly and positively associated with brood size (Table 2; Fig. 1). The same positive significant association was found for the abundance of *C. simulator* (Table 2). A significant positive association was also found between the abundance of *C. kibunensis* and both brood size and phenology (Table 2). In addition, we found a significant effect of the interaction between medication and fumigation treatments on the abundance of *C. festivipennis* females (Table 2) with a higher abundance in non-fumigated nests occupied by control pairs (non-medicated) than in the rests of the groups (LSD test, all P<0.011). As residuals of this model did not follow a normal distribution, the effect of the treatment on the abundance of *C. festivipennis* was also tested using non-parametric

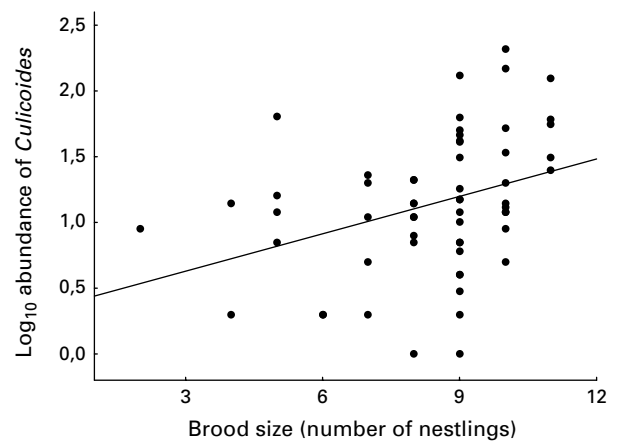


Fig. 1. Relationship between the total abundance of *Culicoides* females (nulliparous, parous and engorged) and brood size in blue tit nests during the spring of 2005 (adjusted R²=0.11, P<0.01). Regression line is shown.

statistics, including 4 treatments, medicated × fumigated nests, control × fumigated nests, medicated × control nests and control × control nests and obtaining the same conclusion (Kruskal-Wallis test: H_{3,59}=11.12, P=0.01), that is that the medicated × fumigation control nests showed a higher

Table 3. Relationship between the abundance of total parous females, total engorged females and *Culicoides* species richness and brood size, hatching date and medication and fumigation treatments

(Significant relationships at $P < 0.05$ are marked in bold. Spearman R (r_s) and adjusted Z (Z) values are shown.)

Dependent variable	Independent variable		
Parous females	Brood size	$r_s = 0.22$	$P = 0.09$
	Hatching date	$r_s = 0.21$	$P = 0.10$
	Medication treatment	$Z = 0.23$	$P = 0.82$
	Fumigation treatment	$Z = 1.62$	$P = 0.11$
Engorged females	Brood size	$r_s = 0.33$	$P = 0.01$
	Hatching date	$r_s = 0.09$	$P = 0.49$
	Medication treatment	$Z = -0.33$	$P = 0.74$
	Fumigation treatment	$Z = 2.85$	$P < 0.01$
Species richness	Brood size	$r_s = 0.26$	$P = 0.047$
	Hatching date	$r_s = -0.08$	$P = 0.54$
	Medication treatment	$Z = 0.05$	$P = 0.96$
	Fumigation treatment	$Z = 2.55$	$P = 0.01$

abundance of *C. festivipennis* than the other groups.

The abundance of nulliparous females was significantly associated with brood size (model: adjusted $R^2 = 0.13$, $P < 0.01$; brood size: $F_{1,57} = 9.40$, $P < 0.01$). In addition, no significant association was found between the abundance of parous females and brood size, phenology or treatments (Table 3). In addition, the abundance of engorged females was significantly higher in nests with larger broods (Table 3). Also, the abundance of engorged females was significantly lower in fumigated nests than non-fumigated nests (Table 3). In addition, no significant association was found between the abundance of engorged females and phenology or medication treatment (Table 3). Finally, a significant and positive association was found between species richness and brood size (Table 3). In addition, although we found that fumigation significantly reduced the species richness in nests (Table 3), no significant association was found between species richness and either phenology or medication treatment (Table 3).

DISCUSSION

Here we report the *Culicoides* infracommunity composition and examine different factors determining their abundance in wild blue tit nests. All the *Culicoides* species found in this study have been previously cited for the Iberian Peninsula (Delécolle, 2002), and 3 of them, *C. festivipennis*, *C. kibunensis* (quoted as *C. cubitalis*) and *C. truncorum* (quoted as *C. sylvarum*) have been previously captured on wild avian hosts (buzzards *Buteo buteo* nests; Votýpka *et al.* 2002; Podlipaev *et al.* 2004). We found that 3 *Culicoides* species had prevalences above 60% and that a very low proportion of nests were free of

biting midges. Because vector abundances may determine the prevalence of blood parasites in their hosts (Sol *et al.* 2000; Yu *et al.* 2000), our results are in accordance with a previous study in the same host population reporting a high prevalence of infection by haemoparasites (Merino *et al.* 2000).

Many, if not all, biting insects have evolved a complex sensory system designed to detect and locate hosts with different receptors including chemo- and visual-receptors (Gibson and Torr, 1999; Grant and Kline, 2003). Blood sucking insects use host-derived odours as cues to detect their hosts (Gibson and Torr, 1999; Mordue, 2003). As shown in electrophysiological studies on several *Culicoides* species, these products are effective in stimulating biting midge receptors (Bhasin *et al.* 2000a; Grant and Kline, 2003; Sollai *et al.* 2007), and their attractive effect on *Culicoides* species has been reported both when they are present on their own (Blackwell *et al.* 1996; Braverman *et al.* 2000; but see Bhasin *et al.* 2000b) and in interaction with other host products (such as CO_2) (Gibson and Torr, 1999; Bhasin *et al.* 2000b; but see Braverman *et al.* 2000). In the case of birds, some of the kairomones responsible for inducing feeding could be the compounds produced by uropygial glands, as previously reported for other blood-feeding arthropods (see Russell and Hunter, 2005 and references therein). For that reason, if more nestlings are capable of producing a higher amount of these products we could expect the pattern obtained here, with higher abundances of *Culicoides* in nests with larger broods. Accordingly, the abundance of *Culicoides* in avian nests increased with nestling age (a correlate of nestling size) (Tomás *et al.* 2008a). In a previous study Tomás *et al.* (2008b) reported the effect of other variables (nest size, nestling condition, female infection status, the abundance of other ectoparasites and parental provisioning rates) also affecting the total abundance

of *Culicoides* in avian nests, although they did not find a significant effect of nestling brood mass (a correlate of brood size) on total *Culicoides* abundance. However, the different experimental designs used here could explain discrepancies between the results of the two studies. For example, we captured biting midges with Petri dishes during a period of 2 days, while Tomás *et al.* (2008b) captured *Culicoides* using a piece of plastic tape during one day. In addition, a considerably lower number of *Culicoides* was captured during 2005 (1531 *Culicoides* females) than in 2004 (more than 2300 *Culicoides*) when the study by Tomás *et al.* (2008b) was carried out. Differences in the species composition of *Culicoides* between both studies may also affect results, but unfortunately this information is not available for the study by Tomás *et al.* (2008b). More experimental studies modifying brood size or the concentration of host attractants should be done in avian nests to reveal the actual importance of these cues to host detection by ornithophilic midges.

We also found a significant association between the medication treatment and the abundance of biting midges in avian nests. Although, we did not measure the efficacy of the medication treatment to reduce the parasite load, we can assume an effect of the treatment in reducing the intensity of infection by *Haemoproteus*, the most common blood parasite affecting this population (see for example, Merino *et al.* 2000; Tomás *et al.* 2005; Martínez-de la Puente *et al.* 2007). The results reported here on the effect of the medication treatment increasing the abundance of *C. festivipennis* in avian nests support a previous study conducted in the same population during 2004 (Tomás *et al.* 2008b) where authors found a higher abundance of biting midges in nests occupied by medicated female birds. Also, our results support a previous study where Darbro *et al.* (2007) found that *Culex* mosquitoes are less likely to feed upon birds infected with *Mycoplasma gallisepticum* maintained in captivity. Different possibilities could explain the higher abundance of this insect species in nests attended by medicated birds. One possibility could be that *C. festivipennis* is able to discriminate between heavily or lightly infected hosts and feed preferentially on those with lower intensities of infection as a defensive strategy due to the harm that blood parasites produce to biting midges (Desser and Yang, 1973; Valkiūnas and Iezhova, 2004). Alternatively, it could be possible that the increase in bird provisioning rates associated with the medication treatment (Merino *et al.* 2000; Tomás *et al.* 2007a) help this insect species in host location. The possibility that the medication treatment modifies, at least in part, the release of host odours that are used as cues for host location by midges should be also considered. In this respect, if *C. festivipennis* was more sensitive to those changes, we could expect an absence of any significant effect of bird medication on

the abundance of other *Culicoides* species. More studies to identify the effect of the medication treatment on the abundance of biting midges in avian nests are needed.

On the other hand, although the total abundance of *Culicoides* was not affected by the fumigation treatment, we clearly found an effect of the insecticide on the abundance of engorged females, suggesting that the insecticide reduced the efficiency of blood feeding by midges, probably through their avoidance of the nesting material saturated with insecticide where nestlings were located. Another possibility could be that the treatment killed a certain proportion of the midge population that rendered a reduction in abundance of engorged midges. In fact, this could also be the reason, at least in part, for the lower species richness found in fumigated nests with respect to control nests. In previous studies where we used the same insecticide, a significant reduction in the abundance of other ectoparasites (fleas, mites and blowflies) was found (Tomás *et al.* 2007b; Lobato *et al.* 2008), suggesting that the higher mobility of biting midges with respect to other nest ectoparasites (mites, fleas and blowflies) could explain the differential efficiency of the treatment between nest-dwelling and flying ectoparasites.

Finally, we found that phenology, estimated as hatching date, is an important factor affecting the abundance of some vector species probably due to its association with meteorological conditions. There are many reports on the effects of both meteorological factors and seasonality on *Culicoides* biology in terms of development, adult survival, distribution, abundance and activity rates (Bishop *et al.* 1996; Gerry and Mullens, 2000; Mellor *et al.* 2000; Wittmann *et al.* 2001; Garvin and Greiner, 2003; Sarto i Monteys and Saiz-Ardanaz, 2003; Lysyk and Danyk, 2007; Martínez-de la Puente *et al.* 2009). The relationship between the abundance of *C. kibunensis* and host phenology suggests that early dates in the host breeding season were less favourable for the development of this species. Overall, our results represent the first evidence for different factors affecting the *Culicoides* infracommunity in the nests of wild birds.

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REFERENCES

- Atkinson, C. T., Greiner, E. C. and Forrester, D. J.** (1983). Experimental vectors of *Haemoproteus meleagridis* Levine from wild turkeys in Florida. *Journal of Wildlife Diseases* **19**, 366–568.
- Banbura, J., Blondel, J., de Wilde-Lambrechts, H. and Perret, Ph.** (1994). Why do female Blue Tits (*Parus caeruleus*) bring fresh plants to their nests? *Journal of Ornithology* **136**, 217–221.
- Beckenbach, A. T. and Borkent, A.** (2003). Molecular analysis of the biting midges (Diptera: Ceratopogonidae), based on mitochondrial cytochrome oxidase subunit 2. *Molecular Phylogenetics and Evolution* **27**, 21–35.
- Bennett, G. F.** (1960). On some ornithophilic blood-sucking diptera in Algonquin Park, Ontario, Canada. *Canadian Journal of Zoology* **38**, 377–389.
- Bhasin, A., Moredue (Luntz), A. J. and Mordue, W.** (2000a). Electrophysiological and behavioural identification of host kairomones as olfactory cues for *Culicoides impunctatus* and *C. nubeculosus*. *Physiological Entomology* **25**, 6–16.
- Bhasin, A., Moredue (Luntz), A. J. and Mordue, W.** (2000b). Responses of the biting midge *Culicoides impunctatus* to acetone, CO₂ and 1-octen-3-ol in a wind tunnel. *Medical and Veterinary Entomology* **14**, 300–307.
- Bishop, A. L., McKenzie, H. J., Barchia, M. and Harris, A. M.** (1996). Effect of temperature regimes on the development, survival and emergence of *Culicoides brevitarsis* Kieffer (Diptera: Ceratopogonidae) in bovine dung. *Australian Journal of Entomology* **35**, 361–368.
- Blackwell, A., Dyer, C., Mordue (Luntz), A. J., Wadhams, L. J. and Mordue, W.** (1994). Field and laboratory evidence for a volatile pheromone produced by parous females of the Scottish biting midge, *Culicoides impunctatus*. *Physiological Entomology* **19**, 251–257.
- Blackwell, A., Dyer, C., Mordue (Luntz), A. J., Wadhams, L. J. and Mordue, W.** (1996). The role of 1-octen-3-ol as a host-odour attractant for the biting midge, *Culicoides impunctatus* Goetghebuer, and interactions of 1-octen-3-ol with a volatile pheromone produced by parous female midges. *Physiological Entomology* **21**, 15–19.
- Bowen, M. F.** (1991). The sensory physiology of host-seeking behavior in mosquitoes. *Annual Review of Entomology* **36**, 139–158.
- Braverman, Y. and Chizov-Ginzburg, A.** (1997). Repellency of synthetic and plant-derived preparations for *Culicoides imicola*. *Medical and Veterinary Entomology* **11**, 355–360.
- Braverman, Y., Chizov-Ginzburg, A. and Mullens, B. A.** (1999). Mosquito repellent attracts *Culicoides imicola* (Diptera: Ceratopogonidae). *Journal of Medical Entomology* **36**, 113–115.
- Braverman, Y., Wegis, M. C. and Mullens, B. A.** (2000). Response of *Culicoides sonorensis* (Diptera: Ceratopogonidae) to 1-octen-3-ol and three plant-derived repellent formulation in the field. *Journal of the American Mosquito Control Association* **16**, 158–163.
- Braverman, Y., Boorman, J., Kremer, M. and Delecolle, J. C.** (1976). Faunistic list of *Culicoides* (Diptera, Ceratopogonidae) from Israel. *Cahiers ORSTOM, serie Entomologie medicale et Parasitologie* **14**, 179–185.
- Braverman, Y., Messaddeq, N., Lemble, C. and Kremer, M.** (1996). Reevaluation of the taxonomic status of the *Culicoides* spp. (Diptera: Ceratopogonidae) from Israel and the Eastern Mediterranean and review of their potential medical and veterinary importance. *Journal of the American Mosquito Control Association* **12**, 437–445.
- Bucher, E. H.** (1988). Do birds use biological control against nest parasites? *Parasitology Today* **4**, 1–3.
- Clark, L.** (1991). The nest protection hypothesis: the adaptive use of plant secondary compounds by European starlings. In *Bird-Parasite Interaction, Ecology, Evolution and Behaviour* (ed. Loye, J. E. and Zuk, M.), pp. 204–221. Oxford University Press, Oxford, UK.
- Cowie, R. J. and Hinsley, S. A.** (1988). Timing of return with green vegetation by nesting blue tits *Parus caeruleus*. *Ibis* **130**, 553–559.
- Darbro, J. M. and Harrington, L. C.** (2007). Avian defensive behavior and blood-feeding success of the West Nile vector mosquito, *Culex pipiens*. *Behavioral Ecology* **18**, 750–757.
- Darbro, J. M., Dhondt, A. A., Vermeylen, F. M. and Harrington, L. C.** (2007). *Mycoplasma gallisepticum* infection in House Finches (*Carpodacus mexicanus*) affects mosquito blood feeding patterns. *American Journal of Tropical Medical and Hygiene* **77**, 488–494.
- Delécolle, J. C.** (1985). Nouvelle contribution à l'étude systématique et iconographique des espèces du genre *Culicoides* (Diptera: Ceratopogonidae) du Nord-Est de la France. Ph.D. thesis, Université Louis Pasteur de Strasbourg, 'Vie et Terre', France.
- Delécolle, J. C.** (2002). Ceratopogonidae. In *Catálogo de los Dípteros de España, Portugal y Andorra (Insecta)*. (ed. Carles-Tolrà Hjorth-Andersen, M.), pp. 26–33. *Monografias S.E.A.*, 8, Spain.
- Desser, S. S. and Yang, Y. J.** (1973). Sporogony of *Leucocytozoon* spp. in mammalophilic simuliids. *Canadian Journal of Zoology* **51**, 793–793.
- Downes, J. A.** (1958). The feeding habits of biting flies and their significance in classification. *Annual Review of Entomology* **3**, 249–266.
- Dyce, A. L.** (1969). The recognition of nulliparous and parous *Culicoides* (Diptera: Ceratopogonidae) without dissection. *Journal of the Australian Entomological Society* **8**, 11–15.
- Edman, J. D., Webber, L. A. and Schmid AA.** (1974). Effect of host defenses on the feeding pattern of *Culex nigripalpus* when offered a choice of blood sources. *Journal of Parasitology* **60**, 874–883.
- Fallis, A. M. and Wood, D. M.** (1957). Biting midges (Diptera: Ceratopogonidae) as intermediate hosts for *Haemoproteus* in ducks. *Canadian Journal of Zoology* **35**, 425–435.
- Garvin, M. C. and Greiner, E. C.** (2003). Ecology of *Culicoides* (Diptera: Ceratopogonidae) in southcentral

- Florida and experimental *Culicoides* vectors of the avian hematozoan *Haemoproteus danilewskyi* Kruse. *Journal of Wildlife Diseases* **39**, 170–178.
- Gerry, A. C. and Mullens, B. A.** (2000). Seasonal abundance and survivorship of *Culicoides sonorensis* (Diptera: Ceratopogonidae) at a Southern California dairy, with reference to potential bluetongue virus transmission and persistence. *Journal of Medical Entomology* **37**, 675–688.
- Gibson, G. and Torr, S. J.** (1999). Visual and olfactory responses of haematophagous Diptera to host stimuli. *Medical and Veterinary Entomology* **13**, 2–23.
- Grant, A. J. and Kline, D. L.** (2003). Electrophysiological responses from *Culicoides* (Diptera: Ceratopogonidae) to stimulation with carbon dioxide. *Journal of Medical Entomology* **40**, 284–293.
- Kettle, D. S.** (1995). *Medical and Veterinary Entomology*. 2nd Edn. CAB International, Wallingford, UK.
- Kremer, M.** (1966). Contribution à l'étude du genre *Culicoides* Latreille particulièrement en France. *Encyclopedie d'Entomologie, serie A* **39**, 1–299.
- Lacroix, R., Mukabana, W. R., Gouagna, L. C. and Koella, J. C.** (2005). Malaria infection increases attractiveness of humans to mosquitoes. *PLoS Biology* **3**, 1590–1593.
- Lafuma, L., Lambrechts, M. M. and Raymond, M.** (2001). Aromatic plants in bird nests as a protection against blood-sucking flying insects? *Behavioural Processes* **56**, 113–120.
- Lobato, E., Merino, S., Moreno, J., Morales, J., Tomás, G., Martínez-de la Puente, J., Osorno, J. L., Kuchar, A. and Möstl, E.** (2008). Corticosterone metabolites in blue tit and pied flycatcher droppings: effects of brood size, ectoparasites and temperature. *Hormones and Behavior* **53**, 295–305.
- Lysyk, T. J. and Danyk, T.** (2007). Effect of temperature on life history parameters of adult *Culicoides sonorensis* (Diptera: Ceratopogonidae) in relation to geographic origin and vectorial capacity for Bluetongue virus. *Journal of Medical Entomology* **44**, 741–751.
- Mands, V., Kline, D. L. and Blackwell, A.** (2004). *Culicoides* midge trap enhancement with animal odour baits in Scotland. *Medical and Veterinary Entomology* **18**, 336–342.
- Marquardt, W. C., Demaree, R. S. and Grieve, R. B.** (2000). *Parasitology and Vector Biology*. 2nd Edn. Academic Press, San Diego, USA.
- Martínez-de la Puente, J., Merino, S., Lobato, E., Rivero-de Aguilar, J., del Cerro, S., Ruiz-de-Castañeda, R. and Moreno, J.** (2009). Does weather affect biting fly abundance in avian nests? *Journal of Avian Biology* (in the Press).
- Martínez-de la Puente, J., Merino, S., Tomás, G., Moreno, J., Morales, J., Lobato, E. and García-Fraile, S.** (2007). Can the host immune system promote multiple invasions of erythrocytes *in vivo*? Differential effects of medication and host sex in a wild malaria-like model. *Parasitology* **134**, 651–655.
- Mellor, P. S., Boorman, J. and Baylis, M.** (2000). *Culicoides* biting midges: their role as arbovirus vectors. *Annual Review of Entomology* **45**, 307–340.
- Merino, S., Moreno, J., Sanz, J. J. and Arriero, E.** (2000). Are avian blood parasites pathogenic in the wild? A medication experiment in blue tits. *Proceedings of the Royal Society of London, B* **267**, 2507–2510.
- Mooring, M. S., Fitzpatrick, T. A., Fraser, I. C., Benjamin, J. E., Reisig, D. D. and Nishihira, T. T.** (2003). Insect-defense behavior by desert bighorn sheep. *The Southwest Naturalist* **48**, 635–643.
- Mordue (Luntz), A. J.** (2003). Arthropod semiochemicals: mosquitoes, midges and sealice. *Biochemical Society Transactions* **31**, 128–133.
- Mukabana, W. R., Takken, W., Coe, R. and Knols, B. G. J.** (2002). Host-specific cues cause differential attractiveness of Kenyan men to the African malaria vector *Anopheles gambiae*. *Malaria Journal* **1**, 17.
- Mullens, B. A., Owen, J. P., Heft, D. E. and Soback, R. V.** (2005). *Culicoides* and other biting flies on the Palos Verdes Peninsula of Southern California, and their possible relationship to equine dermatitis. *Journal of the American Mosquito Control Association* **21**, 90–95.
- Mullens, B. A., Cardona, C. J., McClellan, L., Szijj, C. E. and Owen, J. P.** (2006). *Culicoides bottimeri* as a vector of *Haemoproteus lophortyx* to quail in California, USA. *Veterinary Parasitology* **140**, 35–43.
- Mushi, E. Z., Chabo, R. G., Isa, J. F. W., Binta, M. G., Kapaata, R. W. and Bathuseng, T.** (1999). *Culicoides* spp. (Diptera: Ceratopogonidae) associated with farmed ostriches (*Struthio camelus*) in Botswana. *Veterinary Research Communications* **23**, 183–186.
- Petit, C., Hossaert-McKey, M., Perret, P., Blondel, J. and Lambrechts, M. M.** (2002). Blue tits use selected plants and olfaction to maintain an aromatic environment for nestlings. *Ecology Letters* **5**, 585–589.
- Podlipaev, S., Votýpka, J., Jirků, M., Svobodová, M. and Lukeš, J.** (2004). *Herpetomonas ztiplikana* n. sp. (Kinetoplastida: trypanosomatidae): a parasite of the blood-sucking biting midge *Culicoides kibunensis* Tokunaga, 1937 (Diptera: Ceratopogonidae). *Journal of Parasitology* **90**, 342–347.
- Ratnayake, J., Dale, P. E., Sipe, N. G. and Daniels, P.** (2006). Impact of biting midges on residential property values in Hervey Bay, Queensland, Australia. *Journal of the American Mosquito Control Association* **22**, 131–134.
- Russell, C. B. and Hunter, F. F.** (2005). Attraction of *Culex pipiens/restuans* (Diptera: Culicidae) mosquitoes to bird uropygial gland odors at two elevations in the Niagara Region of Ontario. *Journal of Medical Entomology* **42**, 301–305.
- Sarto i Monteys, V. and Saiz-Ardanaz, M.** (2003). *Culicoides* midges in Catalonia (Spain), with special reference to likely bluetongue virus vectors. *Medical and Veterinary Entomology* **17**, 288–293.
- Satta, G., Goffredo, M., Sanna, S., Vento, L., Cubeddu, G. P. and Mascherpa, E.** (2004). Field disinfection trials against *Culicoides* in north-west Sardinia. *Veterinaria Italiana* **40**, 329–335.
- Shelley, A. J. and Coscarón, S.** (2001). Simuliid Blackflies (Diptera: Simuliidae) and Ceratopogonid Midges (Diptera: Ceratopogonidae) as vectors of *Mansonella ozzardi* (Nematoda: Onchocercidae) in Northern Argentina. *Memórias do Instituto Oswaldo Cruz* **96**, 451–458.

- Sol, D., Jovani, R. and Torres, J.** (2000). Geographical variation in blood parasites in feral pigeons: the role of vectors. *Ecography* **23**, 307–314.
- Sollai, G., Solari, P., Masala, C., Crnjar, R. and Liscia, A.** (2007). Effects of avermectins on olfactory responses of *Culicoides imicola* (Diptera: Ceratopogonidae). *Journal of Medical Entomology* **44**, 656–659.
- Tomás, G., Merino, S., Martínez, J., Moreno, J. and Sanz, J. J.** (2005). Stress protein levels and blood parasite infection in blue tits (*Parus caeruleus*): a medication field experiment. *Annales Zoologici Fennici* **42**, 45–56.
- Tomás, G., Merino, S., Moreno, J., Morales, J. and Martínez-de la Puente, J.** (2007a). Impact of blood parasites on immunoglobulin level and parental effort: a medication field experiment on a wild passerine. *Functional Ecology* **21**, 125–133.
- Tomás, G., Merino, S., Moreno, J. and Morales J.** (2007b). Consequences of nest reuse for parasite burden and female health and condition in blue tits, *Cyanistes caeruleus*. *Animal Behaviour* **73**, 805–814.
- Tomás, G., Merino, S., Martínez-de la Puente, J., Moreno, J., Morales, J. and Lobato, E.** (2008a). A simple trapping method to estimate abundances of blood-sucking flying insects in avian nests. *Animal Behaviour* **75**, 723–729.
- Tomás, G., Merino, S., Martínez-de la Puente, J., Moreno, J., Morales, J. and Lobato, E.** (2008b). Determinants of abundance and effects of blood-sucking flying insects in the nest of a hole-nesting bird. *Oecologia* **156**, 305–312.
- Torres-Estrada, J. L. and Rodríguez, M. H.** (2003). Physic-chemical signals involved in host localization and induction of disease vector mosquito bites. *Salud Pública de México* **45**, 497–505.
- Valkiūnas, G. and Iezhova, T. A.** (2004). Detrimental effects of *Haemoproteus* infections on the survival of biting midge *Culicoides impunctatus* (Diptera: Ceratopogonidae). *Journal of Parasitology* **90**, 194–196.
- Votýpka, J., Oborník, M., Volf, P., Svobodová, M. and Lukeš, J.** (2002). *Trypanosoma avium* of raptors (Falconiformes): phylogeny and identification of vectors. *Parasitology* **125**, 253–263.
- Wittmann, E. J., Mellor, P. S. and Baylis, M.** (2001). Using climate data to map the potential distribution of *Culicoides imicola* (Diptera: Ceratopogonidae) in Europe. *Revue scientifique et technique (International Office of Epizootics)* **20**, 731–740.
- Yu, C.-Y., Wang, J.-S. and Yeh, C.-C.** (2000). *Culicoides arakawae* (Diptera: Ceratopogonidae) population succession in relation to leucocytozoonosis prevalence on a chicken farm in Taiwan. *Veterinary Parasitology* **93**, 113–120.
- Zimmerman, R. H. and Turner, E. C., Jr.** (1983). Host-feeding patterns of *Culicoides* (Diptera: Ceratopogonidae) collected from livestock in Virginia, USA. *Journal of Medical Entomology* **20**, 514–519.