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.

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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, CO_2 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

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 CO_2 (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 Haemo-proteus (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, 40x53k74N, 4x01kW, 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^{x1}) 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-f, 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. 2007 b). 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. (2008 a). 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-f 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. (2008 a) 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 stereomicroscope (10r–64r 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
C. simulator	751 (86·4)	76 (35·6)	44 (45·8)	871 (93·2)
C. kibunensis	322 (79·7)	42 (37·3)	9 (11·9)	373 (81·4)
C. festivipennis	121 (61·0)	60 (30·5)	5 (5·1)	186 (62·7)
C. segnis	59 (42·4)	9 (13·6)	7 (11·9)	75 (50·8)
C. truncorum	14 (13·6)	3 (5·1)	2 (3·4)	19 (20·3)
C. pictipennis	2 (3·4)	2 (3·4)	0 (0)	4 (6·8)
C. circumscriptus	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 R^2 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
C. simulator	$R^2=0·13$	P<0·003	Brood size	$F_{1,57}=9·72$	P<0·003
C. kibunensis	$R^2=0·18$	P<0·002	Brood size	$F_{1,56}=12·37$	P<0·001
C. festivipennis	$R^2=0·06$	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
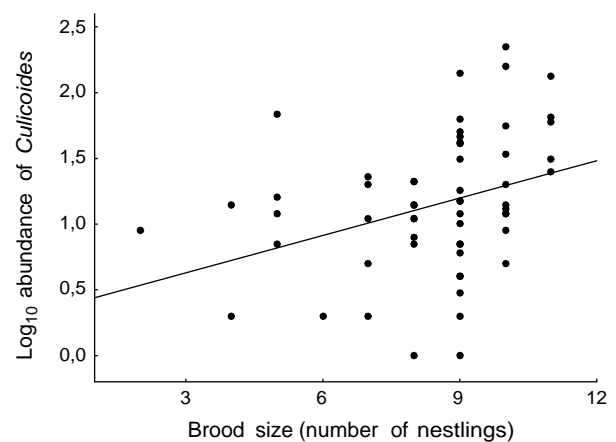

Fig. 1. Relationship between the total abundance of Culicoides females (nulliparous, parous and engorged) and brood size in blue tit nests during the spring of 2005 (adjusted $R^2=0·11$, P<0·01). Regression line is shown.

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; Votỳpka 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. (2008 b) 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. (2008 b) 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. (2008 b). 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. 2008 b) 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. 2007 a) 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. 2007 b ; 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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