Habitat and microclimate affect the resting site selection of mosquitoes

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Abstract. Mosquitoes (Diptera: Culicidae) use certain resting sites during their inactive phase. The microclimatic conditions of these resting sites might affect their physiology and vectorial capacity. In this study, we combined a field and a laboratory study to investigate the natural resting site and temperature preferences of mosquitoes. The field study was conducted at a forest close to Oldenburg (Lower Saxony, Germany) from May to October 2018. Mosquitoes were collected in five different natural habitats with seven replicates each. Temperature was recorded hourly at each site. Significantly more mosquitoes were collected in deadwood (predominantly Culiseta morsitans/fumipennis) and shaded herb layer (predominantly Aedes species) compared to unshaded herb layer or broadleaf and coniferous trees. GLMMs revealed resting site habitats as the best predictor to explain the observed preference patterns, but microclimatic conditions are also involved in mosquito resting site selection. Most mosquitoes were collected at resting sites with relatively colder and more stable temperatures. In concert, laboratory choice experiments with a thermal gradient ring demonstrated that Cs. morsitans/fumipennis avoid temperatures over 30°C. Understanding the small-scaled resting site preferences and the related microclimatic conditions can improve mosquito collection techniques and refine the prediction of mosquito-borne pathogen transmission.

Key words. Culicidae, mosquitoes, natural resting sites, microclimate.

Introduction

Mosquitoes are the most important arthropod vectors of pathogens, particularly in tropical and subtropical regions. In Central Europe, their growing medical importance is demonstrated by the emergence of several mosquito-borne pathogens, including West Nile, Sindbis, and Usutu virus (Scheuch et al., 2018; Ziegler et al., 2020). A key factor for pathogen transmission through mosquitoes is the environmental temperature, affecting mosquito life history traits and pathogen development rate in a mosquito vector (Mordecai et al., 2019). Hence, understanding mosquito-specific resting site and microclimate preferences can refine epidemiological models, which are driven by temperature-dependent history traits and pathogen development rate in the mosquito (Haider et al., 2017).

Adult mosquitoes have species-specific activity patterns over their life span (Clements, 1999). During their inactive phase, mosquitoes use certain resting sites, where they probably spend the majority of the time (Silver, 2008), e.g. to digest blood-meals. This is reflected in the mosquito collections from resting sites commonly consisting of a higher fraction of blood-fed and gravid females compared to conventional baited traps, which target host-seeking females using carbon dioxide as a primary attractant (Burkett-Cadena et al., 2008; Sauer et al., 2020). Various artificial resting sites have been proven as useful tool for mosquito collection, including wooden boxes (Edman et al., 1968), wire-frame shelters (Burkett-Cadena, 2011) or fibre pots (Komar et al., 1995). These artificial resting sites are predominantly utilized by certain mosquito species, particularly of the genera Anopheles, Culex and Culiseta (Morris, 1981; Howard et al., 2011; Burkett-Cadena et al., 2019). Other species are considered to prefer natural resting sites, e.g. members of the genus Aedes (Burkett-Cadena et al., 2008). Mosquitoes seek certain nat-
ural resting site habitats such as understory vegetation, tree cavities, rock crevices or animal burrows, and show strong species-specific preferences for the type of resting site habitat (Service, 1971; Breeland, 1972; Burkett-Cadena et al., 2008; Burkett-Cadena et al., 2013). Nevertheless, the natural resting site preferences of the European mosquito fauna are poorly documented, and the underlying environmental cues for the resting site selection of mosquitoes, in general, are not well understood.

Due to their small size and proportionately large surface area, mosquitoes are likely to be particularly vulnerable to unfavourable climatic conditions (Willmer, 1982). Both temperature and humidity, strongly influence mosquito mortality. As reviewed for *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse), decreasing humidity is associated with an increasing mortality and the temperature-dependent mortality follows a unimodal response with the lowest mortality between 22°C and 27°C (Schmidt et al., 2018). Thus, mosquitoes likely select resting sites with suitable microclimatic conditions to minimize physiological stress during the resting phase. Supporting this assumption, laboratory experiments demonstrated that most mosquito species avoid temperatures above 28°C (Kirby & Lindsay, 2004; Kessler & Guerin, 2008). In addition, different resting site studies in the field documented a higher trapping efficiency in shaded environments (Bidlingmayer, 1971; Service, 1971; Howard et al., 2011). Although these studies did not directly measure microclimatic differences, their results likely indicate a preference for a specific microclimate. Other studies recorded temperatures in a range of potential resting sites to predict the theoretic extrinsic incubation period (EIP) of mosquito-borne pathogens (Meyer et al., 1990; Haider et al., 2017; Thomas et al., 2018). They documented that the temperatures vary up to 10°C and more between different microhabitats, which would strongly influence the temperature-dependent traits of mosquitoes and pathogens. However, it remained unclear, which particular microclimate is most relevant for mosquitoes and if temperatures directly affect the resting site selection.

The aims of this paper were (a) to analyse small-scale resting site preferences for different natural habitats at a forest site and (b) to relate mosquito resting site selection to the habitat associated microclimatic conditions. In order to validate field observations, we conducted complementary laboratory experiments by analysing the temperature preferences of *Culiseta mor- sitans/fumipennis*, which was the most abundant mosquito taxon collected during the field study.

### Material and methods

In 2018, a field study was implemented in an area of 5 hectares in a forest close to Oldenburg (Lower Saxony, Germany) (N53.158°, E8.125°). The area is dominated by mixed oak-hornbeam forest, alder ash forest and spruce forest, and provides various breeding habitats for mosquitoes, including swampy alder forests, woodland ditches or temporary woodland pools.

We selected five types of resting site habitats, representing the most common potential natural resting sites in the forest expected to have different microclimatic conditions: deadwood (root plates of uprooted trees or accumulated remains of forestry), shaded herb layers, unshaded herb layers, broadleaf trees and coniferous trees (Fig. 1). Based on a random block design, seven areas (50×50 m) within the forest were chosen, which each included one replicate of the five different resting site habitats. Thus, in total, we integrated 35 resting sites in the study. Each resting site was equipped with a temperature logger (HOBO Pendant logger UA-001-08, Onset Computer Corporation, U.S.A.), recording hourly data from 3 May to 12 October 2018. Temperature loggers in the herb layer were fastened to a bamboo stick at a height of 0.3 m above the ground. Loggers in the deadwood or trees were fastened within the studied habitats. Resting mosquitoes were collected twice a week during a period from 7 May to 5 July and from 15 August to 12 October 2018 (in total 27 collection days). Each site was sampled 1 minute in a maximum distance of 2 m from the temperature logger. Mosquitoes were collected with a handmade aspirator (Fig. 1) similar to the Prokopack aspirator (Vazquez-Prokopec et al., 2009). Immediately after resting site collection, light intensity (digital lux meter PeakTech 5025, Germany) and wind speed (Floureon Digital Anemometer, U.S.A.) were measured at the position of the temperature loggers. Collected mosquitoes were killed and stored in a freezer at −18°C. Each specimen was identified by morphological characters to the lowest level of taxonomic certainty (Mohrig, 1969; Lechthaler, 2005; Becker et al., 2010).

The statistical significance (P < 0.05) of the differences in the mean temperatures per resting site habitat were tested by an analysis of variance (ANOVA), followed by multiple pairwise comparisons (Tukey HSD test). Each mosquito must have entered the resting site a certain time period before sampling. However, the exact time and duration of resting is unclear. However, artificial resting studies demonstrated that mosquitoes can be collected efficiently within a short sampling rhythm of 24 h or less, indicating, that mosquitoes commonly rest shorter than 24 h (Howard et al., 2011; Brugman et al., 2017; Sauer et al., 2020). Furthermore, Gray et al. (2011) documented that mosquitoes enter resting sites during the morning hours (2 h before and 2 h after sunrise), but not before. We commonly sampled the resting sites between 10.00 and 13.00. Hence, it can reasonably be concluded that the timeframe of 6 h before sampling is of particular relevance for the resting site selection in this study and refers to the time, when mosquitoes actually experienced the resting site temperatures. Thus, we extracted the temperature data from 0 to 6 h before each collection and calculated the mean resting site temperatures per collection as temperature proxy for our statistical models. Four separate GLMMs were applied with the three most abundant taxa (*Ae. annulipes* Group, *Ae. communis* and *Cs. morstians/fumipennis*) and the total number of mosquitoes per resting site collection as response variables, and the resting site habitats (five categories) and resting site temperatures as fixed effects. The seven nested blocks, i.e. the seven sampling areas each with one replicate per resting site habitat, and the collection day were included as random effects. To assess a non-linear response to temperature of the mosquitoes, GLMMs were constructed with resting
site temperatures as a linear and quadratic term, respectively. Hereby, the inclusion of the quadratic term led to lower AICs for all of the four GLMMs and was therefore included in the final models. Considering the overdispersed distribution of the mosquito count data, a negative binomial error distribution was specified with the function 'glmer.nb' of the 'lme4' package (Bates et al., 2015). Variance inflation factors (VIFs) among the explanatory variables, i.e. fixed effects, remained below 2 in all models, indicating no problems with collinearity. Light intensity and wind speed were not included in the models as the punctual measurements are affected by strong natural variation and therefore would have been hard to interpret with respect to mosquito resting site selection. However, these data are provided separately as descriptive values.

In summer 2019, we conducted a laboratory experiment to analyse the temperature preferences of female *Cx. morsitans f. fumipennis*, which were the most common collected taxon in the field study 2018 (Table 1). Mosquitoes were collected in the same area and subsequently exposed to an artificial temperature gradient. In the meantime (maximum: 48 h), collected mosquitoes were kept in a climate chamber (KMF 240, Binder, Germany), simulating a regular summer day of the study area (14 h day: 10 h night with constant 15 °C in the night and linear increase to 25 °C in daylight). Each trial was conducted with one specimen. The temperature gradient was created using a thermal gradient ring (65983-93, PHYWE Systeme, Germany). The device consists of a metal ring (width: 11 cm, outside diameter: 38 cm, inside diameter: 16 cm) surrounded by a rim of 6 cm height (Fig. 2). For this study, the space of action was reduced to a ring with a width of 5 cm and a height of 3 cm. This was necessary, because the thermal gradient ring is only heated at the bottom and commonly used for non-flying insects. The reduced space of action ensures that the mosquito experience the measured temperature at the bottom of the ring.

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**Fig. 1.** Exemplary pictures of the five resting site habitats deadwood (A), shaded herb layer (B), unshaded herb layer (C), broadleaf tree (D), coniferous tree (E) and of the handmade aspirator (F).
Table 1. Number of mosquitoes collected at different resting site habitats during the field study in 2018. The number of blood-fed females are given in brackets.

| Mosquito taxon | Deadwood | Shaded herb layer | Unshaded herb layer | Broadleaf tree | Coniferous tree |
|---------------|----------|------------------|---------------------|----------------|----------------|
|               | Males    | Females (blood-fed) | Males    | Females (blood-fed) | Males    | Females (blood-fed) | Males    | Females (blood-fed) |
| Aedes annulipes Group* | 0 | 242 (5) | 0 | 232 (2) | 0 | 77 (4) | 0 | 10 (0) | 0 | 13 (1) |
| Aedes annulipes (Meigen, 1830) | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| Aedes cantans (Meigen, 1818) | 3 | 0 | 10 | 0 | 3 | 0 | 7 | 0 | 3 |
| Aedes cinerealbigeminus | 4 | 23 (1) | 5 | 20 (1) | 2 | 12 (0) | 0 | 0 | 0 |
| Aedes communis (de Geer 1776) | 25 | 68 (1) | 33 | 104 (3) | 5 | 31 (3) | 2 | 1 (0) | 11 | 15 (0) |
| Aedes rusticus (Rossi 1790) | 1 | 13 (0) | 2 | 10 (0) | 0 | 3 (0) | 0 | 3 (1) | 0 | 1 (0) |
| Aedes vexans (Meigen, 1830) | 0 | 0 | 0 | 1 (0) | 0 | 0 | 0 | 0 | 0 |
| Aedes spp. | 1 | 6 (0) | 0 | 9 (0) | 0 | 6 (0) | 0 | 0 | 0 | 3 (0) |
| Anopheles maculipennis s.l. | 0 | 1 (1) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Anopheles plumbeus (Stephens 1828) | 0 | 1 (1) | 0 | 0 | 0 | 0 | 0 | 0 |
| Culiseta annulata/subochrea | 0 | 1 (1) | 0 | 0 | 0 | 0 | 0 | 0 |
| Culiseta mortitans/fumipennis | 479 | 678 (138) | 2 | 2 (1) | 0 | 2 (1) | 0 | 1 (0) | 3 | 7 (4) |
| Culex pipiens s.l. | 10 | 16 (3) | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 0 |
| Culex modestus Ficalbi, 1890 | 0 | 1 (0) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Unidentified mosquitoes | 2 | 12 (0) | 3 | 0 | 1 | 0 | 2 | 1 (0) | 0 | 0 |
| Total | 525 | 1062 (151) | 59 | 378 (7) | 13 | 132 (8) | 11 | 16 (1) | 17 | 39 (5) |

* Only males of the Aedes annulipes Group were identified to species level.

Furthermore, we enhanced the experimental set-up with a potentiometer (EMD201, Emil Lux, Germany) to regulate the power of the heating plate and a circulating bath with thermo-controller (WKL 26, Haake, Germany) to regulate the water temperature flowing into the device. These modifications allowed the manual adjustability of the temperature gradient. In the course of investigations, two temperature gradients (16 °C to 28 °C and 23 °C to 38 °C) were used to simulate the day temperature range of a regular and a very hot summer day of the field study area. The spatial-temporal stability of the temperatures within the thermal gradient ring was checked several times without mosquitoes. Temperatures were logged (HOBO Pendant logger, see above) in 12 sections every 5 minutes for 5 h. The temperature of six sections pairs were adjusted to similar temperatures (±1 °C). The maximum observed temperature deviation per section was 1.2 °C (Fig. 2). The starting section for mosquito exposition to the thermal gradient ring was selected randomly before each trial. Pictures (resolution: 4000 × 3000) were taken automatically every 20 s for 40 min with a camera (Sony Alpha 6000 with Sony Selp1650 camera lens, Sony, Japan) from 60 cm above the thermal gradient ring (Fig. 2). One specimen showed no movement during the trial and was assumed to be damaged and excluded from the statistical analysis. The final data were based on 39 specimens exposed to the temperature gradient from 16 °C to 28 °C and 10 specimens exposed to the gradient from 23 °C to 38 °C. After each trial, the mosquitoes were killed by freezing (−18 °C) to verify a priori identification of the living specimens. Mosquito position on each image was inspected visually and documented in accordance to the 12 predefined sections (Fig. 2). Based on the 120 images per trial, the percentage...
of observed mosquito positions per section was calculated for each specimen. Hereby, the two sections of the thermal gradient ring with equal temperatures (±1 °C) were summarized (Fig. 2). The fixed effect of temperature on the percentage of observed mosquito positions were analysed separately for the two temperature set-ups (16 °C to 28 °C and 23 °C to 38 °C). Logistic binomial regressions (GLMs) were fit to analyse the proportional response variables.

All statistical analyses and visualization were performed in R version 3.5.1 (R Core Team, 2018), including the packages MASS (Venables & Ripley, 2002), ggplot2 (Wickham, 2016) and dplyr (Wickham et al., 2019).

Results

During the field study in 2018, we collected 2252 mosquitoes of 12 taxa. Among them, 625 were males and 1627 females, including 172 blood-fed females (Table 1). More specimens were collected from May to June (mean 3.9 specimens per resting site) compared to the study period from August to October (mean 0.6 specimens per resting site) (Fig. 3). The number of collected mosquitoes was statistically significant influenced by the type of resting site habitat (Table 3). Most mosquito taxa and specimens were collected in deadwood (Table 1). Culiseta morsitans/fumipennis, the dominant mosquito taxon in this study, was almost exclusively found in resting sites in deadwood habitats (Table 1 and Fig. 4). Specimens of the Ae. annulipes Group and Ae. communis were dominant in the shaded herb layer and deadwood (Fig. 4). Overall, fewer numbers of mosquito specimens and mosquito taxa were collected in broadleaf and coniferous trees (Table 1 and Fig. 4).

Mean temperature differed between the five types of resting site habitat (ANOVA, df(num) = 4, df(denom) = 112 598, F = 68.11, P < 0.001). Pairwise comparisons of the mean temperature showed significant differences for all pairs of habitats providing resting sites, except between broadleaf trees and unshaded herb layers as well as between coniferous trees and broadleaf trees (Fig. 5). The mean temperature difference over the study period was greatest between the unshaded herb layer and deadwood (mean: 0.83 °C, 95% confidence interval: 0.32 °C, Fig. 5). Based on the temperature standard deviation and quantiles per type of resting site habitat, the most stable temperatures were measured in deadwood, whereby the highest temperature amplitudes were observed in the unshaded herb layer (Table 2). The wind speed rarely exceeded 0.1 m/s during our collection days. Light intensity was highest in the unshaded herb layer (Table 2), thus confirming our classification into shaded and unshaded herb layers.

A quadratic relationship was found between resting site temperatures (based on the 6 h timeframe before sampling) and both the number of Ae. communis and the total number of mosquitoes. However, the number of Ae. annulipes Group or Cs. morsitans/fumipennis per resting site was not affected by temperature (Table 3). Overall, there has been a steep decline of the number of collected mosquitoes, when the resting site temperatures exceeded 26 °C (Figure S1). Such mean resting site temperatures (based on the 6 h timeframe before sampling) were solely observed in the unshaded herb layer (Figure S1).

In the laboratory experiment with Cs. morsitans/fumipennis, no statistically significant selection for specific temperature section was observed for the temperature gradient from 16 °C to 28 °C (GLM binomial, z = 0.376, P = 0.707) (Fig. 6A). In contrast, we observed a statistically significant effect on mosquito choice along the temperature gradient from 23 °C to 38 °C (GLM binomial, z = −2.634, P = 0.008), which was expressed as a steep decrease of the percentage mosquito observations with increasing temperatures (Fig. 6B).

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Fig. 4. Mean (with 95% confidence intervals) number of mosquitoes per resting site collection for the *Ae. annulipes* Group, *Ae. communis*, *Cs. morsitans/fumipennis* and all mosquito taxa given for five different resting site habitats: C, coniferous tree; DW, deadwood; HL+, shaded herb layer; HL−, unshaded herb layer, L, broadleaf tree.

Fig. 5. Tukey’s multiple pairwise comparisons of the mean temperatures differences between the five resting site habitats. The temperature data refers to the study period from May to October 2018. Confidence intervals that intersect the dashed line at 0 level indicate non-significant differences between a pair of resting site structures. Abbreviations: C, coniferous tree; DW, deadwood; HL+, shaded herb layer; HL− = unshaded herb layer; L, broadleaf tree.

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**Table 2.** Abiotic parameters recorded in five resting site habitats. Temperature was logged hourly. Light intensity and wind speed was recorded manually after each resting site sampling.

| Abiotic parameter                  | Deadwood | Shaded herb layer | Unshaded herb layer | Broadleaf tree | Coniferous tree |
|------------------------------------|----------|-------------------|---------------------|----------------|-----------------|
| Mean temperature (SD) [°C]         | 16.45(4.96) | 16.79(5.51)      | 17.28(7.11)        | 17.14(5.72)   | 17.10(5.77)     |
| Maximum temperature (95% quantile) [°C] | 41.22(24.93) | 41.34(26.39) | 50.46(30.86)      | 38.27(27.17)  | 37.16(27.08)    |
| Minimum temperature (5% quantile) [°C] | 1.65(8.68)    | 0.78(8.38)       | −1.57(7.58)        | 0.89(8.28)    | 1.11(8.08)      |
| Mean light intensity (SD) [lux]    | 947(1164)    | 2180(5918)       | 16792(23 438)      | 1394(4682)    | 2705(6785)      |
| Mean wind speed (SD) [m/s]         | 0.02(0.16)    | 0.03 (0.19)      | 0.02 (0.19)        | 0.01 (0.07)   | 0.01 (0.08)     |

**Table 3.** Parameter estimates of the fixed effects in the GLMMs for the three most abundant mosquito taxa and the total number of mosquitoes per resting site (rs) collections.

| Response                                      | Collection days* | Fixed effects      | Coefficient | SE    | z-Value | P-value |
|-----------------------------------------------|------------------|--------------------|-------------|-------|---------|---------|
| *Ae. annulipes* Group per rs collection       | 20               | (Intercept)        | 0.398       | 0.501 | 0.793   | 0.428   |
|                                               | 20               | Shaded herb layer  | −0.990      | 0.225 | −4.404  | <0.001  |
|                                               | 20               | Broadleaf tree     | −2.428      | 0.337 | −7.211  | <0.001  |
|                                               | 20               | Coniferous tree    | −2.340      | 0.331 | −7.242  | <0.001  |
|                                               | 20               | Deadwood           | 0.101       | 0.195 | 0.515   | 0.606   |
|                                               | 20               | rs Temperature ²   | −0.002      | 0.001 | −1.342  | 0.180   |
| *Ae. communis* per rs collection              | 11               | (Intercept)        | 2.316       | 0.774 | 2.993   | 0.003   |
|                                               | 11               | Shaded herb layer  | −0.942      | 0.251 | −3.746  | <0.001  |
|                                               | 11               | Broadleaf tree     | −3.364      | 0.607 | −5.539  | <0.001  |
|                                               | 11               | Coniferous tree    | −1.362      | 0.272 | −5.539  | <0.001  |
|                                               | 11               | Deadwood           | −0.298      | 0.200 | −1.490  | 0.136   |
|                                               | 11               | rs Temperature ²   | −0.007      | 0.002 | −3.525  | <0.001  |
| *Cs. morsitans*/*fumipennis* per rs collection| 21               | (Intercept)        | −4.220      | 1.003 | −4.206  | <0.001  |
|                                               | 21               | Shaded herb layer  | −0.927      | 0.986 | −0.939  | 0.348   |
|                                               | 21               | Broadleaf tree     | −1.216      | 1.174 | −1.036  | 0.300   |
|                                               | 21               | Coniferous tree    | 1.073       | 0.717 | 1.496   | 0.135   |
|                                               | 21               | Deadwood           | 5.553       | 0.607 | 9.149   | <0.001  |
|                                               | 21               | rs Temperature ²   | −0.001      | 0.002 | −0.332  | 0.740   |
| Total no. of mosquitoes per rs collection     | 24               | (Intercept)        | 0.881       | 0.493 | 1.786   | 0.074   |
|                                               | 24               | Shaded herb layer  | −0.984      | 0.188 | −5.226  | <0.001  |
|                                               | 24               | Broadleaf tree     | −2.599      | 0.218 | −9.437  | <0.001  |
|                                               | 24               | Coniferous tree    | −1.757      | 0.218 | −8.052  | <0.001  |
|                                               | 24               | Deadwood           | 1.470       | 0.161 | 9.114   | <0.001  |
|                                               | 24               | rs Temperature ²   | −0.003      | 0.001 | −2.164  | 0.031   |

*Only collection days with more than 3 specimens per analysed taxon were included in the GLMMs. Significant P-values are given in boldface (< 0.05).*

**Discussion**

Our results confirm species-specific preferences of mosquitoes for resting in different natural habitats. The highest number of mosquitoes were collected in deadwood, which is mainly due to the specific resting site preferences of the dominating taxon *Cs. morsitans*/*fumipennis* (99% were collected in deadwood). However, other species were observed to prefer other habitats, e.g. specimens of the genus *Aedes*, which were found in high numbers in the shaded herb layer. These results are in line with previous resting site studies, showing that *Aedes* commonly prefer to rest in the understory vegetation (Bidlingmayer, 1971; Service, 1971; Burkett-Cadena et al., 2008). On the other hand, branches and vegetation of broadleaf and coniferous trees are rather unattractive resting sites for most mosquito species, which is in accordance with the studies by Service (1971) and Kay (1983). The collected taxa represent a typical mosquito community for wet forests in Central Europe (Becker et al., 2010). The observed preferences for root plates of uprooted trees and other deadwood accumulations might be characteristic for woodland species, as these habitats naturally occur in forest ecosystems and are frequently found across most forest types (Travaglini et al., 2007). They provide shelter from unsuitable weather conditions (e.g. wind or precipitation) and more stable temperatures compared to other resting site habitats. However, resting site temperatures alone are not sufficient to explain the preferences for deadwood. Considering the clear resting site preferences of *Cs. morsitans*/*fumipennis*, further environmental cues probably influence mosquito resting site selection. For *Cs. morsitans*/*fumipennis*, we hypothesize that the selection is triggered by the dark cavities which are present in deadwood. The preference of *Cs. morsitans*/*fumipennis* for dark cavities is underpinned by a previous study, which demonstrated garden pop-up bags as useful artificial resting sites to collect *Cs. morsitans*/*fumipennis* (Sauer et al., 2020). A study from the tropics further supports this suggestion, showing...
that species communities collected in buttress roots and hollow trees are similar to artificial resting sites, but differ from the mosquito community collected in the understory vegetation (Burkett-Cadena et al., 2013). Similar to the deadwood, the black inside of artificial resting sites is characterized by a clear optical contrast to the background, potentially providing a visual cue for the resting site selection of certain mosquito taxa (Bentley et al., 2009).

Although the GLMM-statistics indicated the type of resting site habitat as best predictor to explain the observed patterns, resting site temperatures have an impact on the resting site selection of mosquitoes. In this study, mosquitoes tend to select habitats providing resting sites with a more stable microclimate. Under warm and dry conditions, mosquitoes avoid high temperatures in the unshaded herb layer and select habitats with relative colder temperature conditions, in particular in deadwood habitats or in the shaded herb layer. This avoidance of high temperatures was most obvious for the collected Aedes species, which were frequently found in herb layer and deadwood habitats. In contrast, Cs. morsitans/fumipennis was almost exclusively collected in deadwood habitats irrespective of the resting site temperatures. However, the laboratory experiments showed that Cs. morsitans/fumipennis avoid temperatures above 30°C.

In line with other laboratory choice experiments, mosquitoes (here: Anopheles, Culex and Aedes) prefer cold and humid over warm and dry microclimates (Kessler & Guerin, 2008). Similarly, experiments with a continuously heated cage demonstrated that Anopheles gambiae s.s. and Anopheles arabiensis leave the cage at temperatures greater than 33°C and 35.7°C, respectively (Kirby & Lindsay, 2004). Taken together, the results of our field and laboratory observations suggest that mosquitoes avoid unfavourable microclimates, in particular, when temperatures exceed 30°C.

The avoidance of high temperatures probably reduces physiological stress during the resting phase. Physiological fitness parameters, e.g. lifespan and relative fecundity, of vector species, such as Ae. aegypti and Ae. albopictus, rapidly decreases at a temperature above 30°C (Schmidt et al., 2018; Mordecai et al., 2019). Hence, understanding mosquito temperature preferences might be important for epidemiological models, which predict temperature-dependent traits of mosquitoes and pathogens. These models commonly use temperature data from standardized weather stations (Mordecai et al., 2017) or remote sensing (Parselia et al., 2019). Haider et al. (2017) demonstrated that these data do not necessarily reflect the microclimatic conditions of potential habitats where mosquitoes select resting sites. In agreement with the present study, the authors recorded strong small-scaled temperature variations between different resting site habitats, which would strongly affect the predicted pathogen development rate in a vector. Considering the observed resting site preferences, standardized meteorological data would probably overestimate the actual temperatures experienced by the mosquitoes, in particular at warm and dry days, when mosquitoes seek for cool, protected resting sites. However, our conclusions are limited to the environment and mosquito species under study. Further habitats should be analysed to quantify temperature difference between species-specific resting sites and standardized meteorological data. In addition, the mean resting duration and movement between resting sites needs further investigation to adequately interpret the importance of the resting site temperatures during the lifespan of a mosquito.

Besides temperature, relative humidity can affect mosquito physiology (Schmidt et al., 2018) and activity (Gray et al., 2011). Although we did not directly measure humidity, the shaded herb layer and the deadwood habitat should be associated with a higher and more stable relative humidity in contrast to the other analysed habitats. This, in turn, may favour mosquito resting site selection.

In conclusion, our results demonstrate explicit natural resting site preferences of mosquitoes. Highest number of specimens and species were collected in resting sites at deadwood and the shaded herb layer. In addition, our field and laboratory results suggest that small-scale temperature differences contribute to mosquito resting site selection and in particular to avoid of resting site temperatures above 30°C.
Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Number of mosquitoes per resting collection for the Ae. annulipes Group, Ae. communis, Cs. morsitans/fumipennis and all mosquito taxa given for the mean resting site temperatures based on 6 h before each collection.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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