Feeding Site Specificity in Frog-biting Midges (Corethrellidae)

Jonas Virgo · Thomas Eltz

Abstract Hematophagous arthropods often choose predictable feeding sites on their hosts´ body, presumably to maximize blood uptake while minimizing costs. Feeding sites can be host-specific, mediated by intrinsic host characters and/or specific preferences of the blood feeder. We investigated feeding site specificity in a community of frog-biting midges (*Corethrella* spp.) and frog hosts in La Gamba, Costa Rica. Midge distribution on hosts differed significantly between 12 investigated frog species, indicating that intrinsic host properties influence potential feeding sites. However, realized feeding sites were also significantly different between four investigated *Corethrella* morphotypes, across all hosts but also within certain shared hosts, indicating feeding site partitioning among *Corethrella*. We propose that the diversity of feeding sites in *Corethrella*-frog associations is determined by an interaction of host traits, e. g. calling site, defensive behavior or skin thickness, and midge traits, especially body size and corresponding mouthpart size/structure.

Keywords Corethrellidae · host specificity · hematophagy · feeding site

J. Virgo (✉) · T. Eltz
Department of Animal Ecology, Evolution
and Biodiversity, Ruhr-Universität Bochum,
Universitätsstr. 150, 44801 Bochum, Germany
e-mail: Jonas.virgo@rub.de

Introduction

Differential feeding-site specificity has been described for a broad range of both permanent and periodic blood-sucking arthropods (see Lehane 2005). Biting often occurs on body areas that facilitate blood uptake due to certain morphological host traits, such as skin thickness and density of hairs/feathers (e. g. Mullens and Gerhardt 1979). Blood-feeders themselves are constrained by morphological features such as the size and structure of mouthparts (Krenn and Aspöck 2012) or adhering structures such as claws and tufts (Haarløv and Haarlov 1964). Furthermore, numerous ecological and behavioral traits on both sides may determine feeding associations, shifting the tradeoff between a minimal energetic expenditure for blood sucking and the evasion of host defenses (e. g. active repellent movements or grooming; Edman et al. 1972; Murray 1987).

Here, we investigate feeding site specificity among a community of frog-biting midges (Diptera: Corethrelidae) and their frog hosts in La Gamba, Pacific Costa Rica. Female *Corethrella* eavesdrop on the mating calls of male frogs, their primary blood hosts (Camp and Irby 2017; McKeever and Hartberg 1977). Despite a rather generalist acoustic foraging behavior (Grafe et al. 2008; Virgo et al. 2019), midges partition among the host community by using unknown (but see da Silva and Breviglieri 2021) close-range recognition cues (Virgo et al. 2021; also see Grafe et al. 2019). Further observations indicate yet unrevealed

levels of specificity, as realized feeding sites on the host body may vary between frog species (Borkent 2008; Borkent and Grafe 2012; De Silva et al. 2014) and possibly among midge species (this paper).

Based on midge catches from frog hosts collected over three years, we tested whether feeding site specificity exists in frog-biting midges and evaluated whether it is mediated by variable properties of frog hosts or variation of biting preferences among species of *Corethrella*, or both.

Methods

The data represents a subset of midges collected for a study on *Corethrella* host specificity at the La Gamba research station in the Golfo Dulce area, Pacific Costa Rica (Virgo et al. 2021), for which we had recorded information on feeding site (2018 to 2020). Female *Corethrella* spp. were collected during blood uptake from frogs with a handheld aspirator. We recorded the feeding site, differentiating between four feeding site categories: (1) nostrils, (2) head and dorsum, (3) hindlegs, and (4) toes (Fig. 1). Counts are based on the specimens collected. Note that on some occasions observed midge infestation on hosts was higher than realized catches, as not all feeding individuals could be collected due to flight-proneness (of either host or midge). However, this appeared unrelated to any particular feeding site.

Corethrella morphotypes were assigned based on morphological features, neglecting cryptic species diversity in *C. ranapungens* and *C. amazonica/C. ramentum* species groups (see Virgo et al. 2021). We took morphometric measurements for a subset of preserved midges (EtOH) using an Olympus SZX16 stereomicroscope, ColorviewIII camera and Cell^D software (Olympus Corporation, Tokio, Japan). We performed Fisher´s exact test on count data of frogs and midges to test for partitioning of hosts and feeding sites in *Corethrella*. We used Pearson correlation test to assess the effect of total midge catches on observed host and feeding site diversity. Statistical analyses were performed in R (R Core Team 2017).

Results and Discussion

We recorded feeding sites for 507 midges from 229 individual frogs (12 species of frogs; Table 1).

Most midges were collected from the nostrils (54%), followed by the hindlegs (28%), the head and dorsum

Fig. 1 Female *Corethrella* spp. biting male frogs, showing differences in feeding site selection. **A** *Corethrella* spp. aggregating around the nostrils of *Incilius coniferus*; **B** *Corethrella* spp. aggregating in patches on head and thoracic dorsum of *Smilisca phaeota*; **C** *Corethrella* spp. aggregating on hind legs of *Scinax boulengeri*; **E** *Corethrella* spp. approaching and biting toes of *Dendropsophus ebraccatus*

Table 1 Distribution (total catch numbers) of frog-biting midges (*Corethrella* spp.) collected from frog hosts in La Gamba, Costa Rica. We categorized four distinct feeding sites: (1) Nostrils, (2) head/dorsum, (3) hindlegs, and (4) toes. *Data includes double counts for frogs attacked by multiple *Corethrella* spp. simultaneously; the number of individually sampled frogs was 229. Measurements (thorax + abdomen) of *Corethrella* spp. were taken under a dissecting microscope in EtOH on undissected specimens. The observed perch sites of calling male frogs are presented (a: aquatic, partly submerged; tg: terrestrial, ground level; te: terrestrial, elevated; **stream-associated breeder, sitting on streambed rocks)

Corethrella - host association	N frogs	N midges				
Corethrella ranapungens (1.34 mm, σ=0.1, N=10)	**144**	**337**	**274**	**24**	**29**	**10**
Incilius coniferus (a)	11	75	75	0	0	0
Rhinella marina (tg)	2	6	1	5	0	0
Engystomops pustulosus (a)	5	8	8	0	0	0
Leptodactylus savagei (tg)	1	4	4	0	0	0
Agalychnis callidryas (te)	3	3	1	1	1	0
Boana rosenbergi (a, te)	6	12	9	2	1	0
Dendropsophus ebraccatus (te)	10	13	0	3	0	10
Scinax boulengeri (te)	36	61	22	13	26	0
Scinax elaeochrous (te)	69	154	154	0	0	0
Smilisca phaeota (a, te)	1	1	0	0	1	0
Corethrella peruviana (1.96 mm, σ=0.09, N=7)	**93**	**151**	**2**	**42**	**107**	**0**
Agalychnis callidryas (te)	4	4	0	3	1	0
Boana rosenbergi (a, te)	11	21	0	8	13	0
Scinax boulengeri (te)	54	94	1	10	83	0
Smilisca phaeota (a, te)	2	5	0	2	3	0
Smilisca sordida (tg**)	22	27	1	19	7	0
Corethrella sp. LG1 (1.65 mm, σ=0.09, N=6)	**7**	**13**	**0**	**1**	**0**	**12**
Boana rosenbergi (a, te)	2	8	0	0	0	8
Dendropsophus ebraccatus (te)	1	1	0	0	0	1
Scinax boulengeri (te)	3	3	0	0	0	3
Smilisca sordida (tg**)	1	1	0	1	0	0
Corethrella amazonica / C. ramentum (1.8 mm, σ=0.24, N=5)	**6**	**6**	**0**	**1**	**4**	**1**
Leptodactylus insularum (tg)	1	1	0	0	1	0
Smilisca sordida (tg**)	5	5	0	1	3	1
Total:	**250***	**507**	**276**	**68**	**140**	**23**

(13%), and the toes (5%). We found no significant correlation between the number of observed feeding sites per frog species and the total number of midge catches (r=0.18, *p*=0.57, t=0.58, df=10), or host individuals (r=0.34, *p*=0.29, t=1.13, df=10). The initial landing site often differed from the realized feeding site, which was then consecutively approached by walking (J. Virgo, pers. obs.). During this orientation we observed no probing with the proboscis; instead, midges appeared to use non-invasive mechanisms and unknown host cues to identify suitable feeding areas (see also De Silva et al. 2014). Note that in no case were there any midges collected from the front legs or the ventral body side, probably because these body parts are less accessible and/or better defended. Anecdotal observations suggest that the type and amount of defense movements (e. g. kicking with legs, swatting,

submerging in water) vary between host species (J. Virgo, pers. obs.), however, this has not been further investigated. Observed feeding sites differed significantly among the investigated frog species (p < 0.001; N=507; df=33), indicating that host properties affect the choice of feeding site in *Corethrella*. Among the better represented host species (catch numbers > 10) there were conspicuous differences in midge distribution (Table 1). On the Green Toad *Incilius coniferus* (75 midges collected from 11 frogs) and the tree frog *Scinax elaeochrous* (154 from 69) all midges were aggregated around the nostrils. In contrast, on *Scinax boulengeri* midges were mostly (69% of catches) feeding at the hindlegs (109 midges from 52 frogs). In two other species of tree frogs the dorsal head and thorax (*Smilisca sordida*) or the toes (*Dendropsophus ebraccatus*) were targeted most often. In the tree frog *Boana*

rosenbergi, midges were spread more evenly across the body, perhaps showing a slight preference for the hindlegs.

These findings are consistent with de Silva et al. (2014) who found that feeding sites of midges differed between Tungara frogs (*Engystomops pustulosus*) and two species of tree frogs in Panama. Differences were suggested to arise from differences in the vascular properties (blood capillary density, size, and depth) of their skin between different frog species (De Silva et al. 2014). While this is certainly plausible, we suggest that other, more simple mechanisms may also apply: In frog species calling from water, e. g. *I. coniferus* (this study) and *E. pustulosus* (De Silva et al. 2014), nostrils may simply be the highest and therefore safest and least disturbed spot on the host (see information on frog perch sites in Table 1). Other observations support this hypothesis: The Gladiator frog *Boana rosenbergi* was frequently observed calling from either terrestrial perches or from water. While midges were distributed across the whole body on terrestrial callers, feeding was limited to the nostrils when frogs were calling from the water (J. Virgo, pers. obs.). However, simple accessibility cannot explain exclusive biting at the nostrils of the tree frog *S. elaeochrous* calling from leaves above water. In this species also fully exposed frogs carried midges exclusively around their nostrils.

Different midge species could also differ in their biting site preferences, due to, e. g., differences in mouth part morphology and/or specific orientation behavior. Observed structural differences of mouthparts (e. g. the shape and size of the mandibular teeth, Borkent 2008; McKeever 1986) could be associated with different hosts or could reflect different feeding strategies among shared hosts. This had also been suggested by De Silva et al. (2014), who found that the length of the labium differed significantly among a set of *Corethrella* spp. in Panama. However, quantitative data of feeding site differentiation between midge species were not presented. Among the midges analyzed in the present study, we identified four *Corethrella* morphotypes in different proportions: *C. ranapungens* was most abundant (N=337; 66%), followed by *C. peruviana* (151; 30%), *C. sp. 'LG1'* (13; 3%), and *C. amazonia/ C. ramentum* (6; 1%). Observed feeding sites were significantly different for the four *Corethrella* morphotypes (p<0.001, N=507, df=9): *C. ranapungens* was predominantly collected from the nostrils (81% of catches, observed in 8 of 10 host species), followed by the hindlegs (9%),

head and thorax (7%), and toes (3%). *C. peruviana* was mostly collected from the hindlegs (71%, observed in 5 of 5 host species), followed by head and thorax (28%) and nostrils (1%). *Corethrella sp. 'LG1'* was almost exclusively found feeding on the toes, usually approaching from the substrate (92%, observed in 3 of 4 host species). The most rarely sampled *C. amazonica/C. ramentum* was collected from the hindlegs (66%), head/thorax (17%), and toes (17%) of two host species. Overall, *C. ranapungens* made up >99% of nostril-catches, whereas proportions shifted towards the remaining feeding sites in the other morphotypes (dorsum: *C. peruviana* >62%; hindlegs: *C. peruviana* >76%; toes: C. sp. LG1 >52%).

Feeding site distributions were significantly different on different hosts for the two most frequently collected midges, *C. ranapungens* and *C. peruviana* (both p<0.001). This suggests an interaction between midge-specific and host trait-related effects on feeding site selection. Such an interaction could be based on differences in midge body size, especially related to mouthpart size and robustness. For example, smaller midge species with shorter or less robust mouthparts could be more constrained in their feeding site selection on some frog species. Indeed, *C. ranapungens* is the smallest of the four investigated species (Table 1; also compare Borkent 2008), possibly confining it to the soft and accessible tissues around the nostrils. More detailed examinations will be needed to explore the relationship between size, mouthpart morphology and feeding site across a larger diversity of frog-biting midges.

The number of *Corethrella* spp. collected varied from 1–3 between frog species and showed no significant correlation with the total number of midges collected (r=0.2, p=0.53, t=0.66, df=10). Feeding site distributions of multiple *Corethrella* spp. on shared host species varied significantly in *S. boulengeri* and *B. rosenbergi* (both p<0.001), the two frog hosts with the highest number of sampled midges. This indicates host partitioning in midges on shared frog species and further supports the idea of diversified feeding site preferences among *Corethrella* spp.. On a different note, midges appeared to aggregate in groups on their hosts (see Fig. 1b), suggesting that the presence of conspecific and/or heterospecific midge individuals guides feeding site selection of others. Finally, it is unknown how often midges take up blood during their life, and whether individual learning is involved in host or feeding site selection (see McCall and Kelly 2002; Mwandawiro et al. 2000).

To conclude, our data show significant feeding site specificity of *Corethrella* frog-biting midges in a neotropical frog community. Feeding site selection appears to be related to host properties (e. g. skin thickness, calling behavior, defense reactions) but it is also differentiated among midge taxa. Midge size, in conjunction with mouthpart size and robustness, may be the simplest explanatory variable for variation among midge species.

Declarations

Competing Interests The authors have no competing interests to declare that are relevant to the content of this article.

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