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Estimating the mean abundance and feeding rate of a temporal ectoparasite in the wild: *Afrocimex constrictus* (Heteroptera: Cimicidae)

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Abstract

The feeding frequency of blood-feeding invertebrates in the wild is largely unknown but is an important predictor for the potential of disease transmission and for estimating the effects blood feeding may have on the host population. We present a method to estimate the mean feeding frequency per individual parasite from the frequency distribution of fed and unfed individuals in the wild. We used three populations of the cimicid species, *Afrocimex constrictus*, that parasitises the fruit bat *Rousettus aegyptiacus*. We found that the area occupied by a bug refugium was a good predictor of the number of bugs in that refugia. The estimated parasite population sizes ranged from ca. 25,000 to 3 million bugs. Their mean abundance was 1–15 bugs per host individual. Preventing feeding by bugs in their natural habitat showed that bugs took approximately 20 days to return to an unfed stage. A formula is presented by which the distribution of digestion stages in the samples was used to calculate that *A. constrictus* feeds approximately every 7–10 days. The dry weight of a full blood meal was approximated as 13.3 mg. Therefore *A. constrictus* is estimated to draw an average of 1–28 μL blood per host per day. We suggest that any of our methods can be adjusted to be used in other haematophagous insects to estimate host and parasite population size, mean parasite abundance and blood meal size as well as mean feeding frequency in the wild, including the bed bug species that parasitise humans.

Keywords: Blood feeding: Ectoparasite; Feeding frequency; Haematophagy; Mean abundance

1. Introduction

Many insect-borne diseases are transmitted while an infected vector takes blood from the host (e.g., Lehane, 2005). Therefore, the average number of parasites per host individual (the mean abundance *sensu* Bush et al., 1997) and the number of times a vector insect feeds on a host are important predictors of disease prevalence in the host population (vectorial capacity – Burkot, 1988; Smith et al., 2004; Lehane, 2005). In addition, the feeding rate of vector insects can also be used to predict how human, livestock and wildlife hosts suffer from blood loss, allergic reactions to the vector's saliva introduced during each bite and the extent of secondary infections (Usinger, 1966; Marshall, 1981; Axtell, 1999; Brown and Brown, 1996; Reinhardt and Siva-Jothy, 2007).

Despite its importance, the feeding rate in the wild is unknown for many, if not most, haematophagous invertebrates. For example, while the common bed bug, *Cimex lectularius*, feeds every week in laboratory studies (e.g., Siva-Jothy, 2006; Usinger, 1966) such figures often do not mimic field conditions such as the degree of host availability, temperature regimes and host behaviour (Marshall, 1981; Reinhardt and Siva-Jothy, 2007). Their applicability in the field may, therefore, be limited. Other studies use so-called human landing assays (e.g., Shililu et al., 2004; Almeida et al., 2005). In those the impact of the biting is

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estimated from the number of mosquitoes per day landing on human subjects. This method cannot be employed under a range of circumstances, and not for blood sucking insects other than Diptera. In this paper we suggest a method to estimate the average frequency by which an individual host is being bitten using the mean abundance of the parasite, the proportion of fully blood-engorged parasite individuals (i.e., those that have had a recent blood meal) and the time it takes to digest the blood meal, i.e., for fully engorged individuals to return to the non-fed stage. We illustrate this method using the temporary ectoparasite *Afrocimex constrictus* Ferris and Usinger, a cimicid species that parasitises fruit bats.

2. Materials and methods

In February 2004 and March 2005 *A. constrictus* was investigated at three caves in Mount Elgon National Park, Kenya, where the species has resided for at least 20 years (Kock, 1987). The three caves – Kitum, Makingeny and Ngwarisha – are also known as elephant caves because elephants visit them in order to obtain salt. The caves and their geomorphology are described in detail by Lundberg and McFarlane (2006). These caves also harbour very large populations of the Egyptian fruit bat, *Rousetta aegyptiacus* which is the only cave-dwelling fruit bat occurring in Kenya (Kingdon, 2003). In all three caves, the host species was superdominant (>95% of the individuals) which, therefore, largely precludes differences in the parasites' feeding status caused by different host species (Marshall, 1981; Krasnov et al., 2003).

Parasites were stored in 96% ethanol. They were counted, sexed and their digestion stage classified in our laboratory at Sheffield, UK. Size classes could not be used to distinguish instars but last instars are identifiable from their adult-like size. Adults were sexed by the presence or absence of the male paramere (see Usinger, 1966).

We first estimated the host population size. In Kitum we estimated in the field and from a photograph that bats occupied less than 5×5 cm of space when in their day roost, i.e., there were ca. 400 bats/m^2 (see also Kulzer, 1979 for the notion of their close aggregation). Using torch lights, we estimated the total area occupied by roosting bats as derived from the area that was illuminated by the reflecting lights from the fruit bats' eyes.

Second, we estimated the parasite population size. In 2004 we collected all individuals of all stages of five entire refugia in each of the three caves. A refugium is a distinct patch of aggregated bugs (Fig. 1). A sixth sample in each cave was a random collection of individuals moving about the cave walls or on the ground. In order to increase the variation in refugia size, we additionally sampled two large and three small refugia in 2005. Each of the refugia was photographed with a scale reference (Fig. 1). The digital images were imported into an image analysis system (Optimas 6.1, Optimas Corporation), the area occupied by bugs (Fig. 1) was measured and refugium size directly calculated. We then counted the number of bugs in each of the refugia and employed regression methods in order to find a relationship between the number of individuals in a refugium and its size. We also calculated the mean number of bugs per refugium (mean density).

In 2004, we estimated the total area infested by bugs in each cave. In 2005, all cave walls in Makingeny and Ngwarisha on which bugs had gathered were photographed. The inclusion of the ruler in the photograph allowed us to estimate the size of the refugium patches using the image analysis system. We then used the mean density of bugs per refugium in order to project the entire parasite population size.

We estimated the feeding frequency in the field using differences in the digestion stage between parasite individuals. We also estimated the rate by which the blood meal was digested. Using a general decay rate we were able to

Fig. 1. Refugia of *Afrocimex constrictus* on a cave wall. The inset shows a single refugium together with a ruler.

calculate the feeding frequency as follows. In its most general form, decays are described by exponential functions whereby the amount N at time t equals the original amount N_0 multiplied by a decay rate b

$$N(t) = N_0 * e^{-b*x} \quad (1)$$

where x is the number of time units since N_0 happened. In the case of a linear decline, the amount $N(t)$ at time t can be described as

$$N(t) = N_0 - (x * b) \quad (2)$$

Here, fully engorged individuals represent the amount N_0, whereas the digestion stage of individuals randomly collected in the field represent N at time t.

We classified four different digestion stages. Stage 0 comprised individuals that were not fed, their cuticle was almost translucent and their bodies were flat. Stage 3 were individuals that were fully fed. Their bodies were cylindrical rather than flat and very dark from the recent blood meal. Easily distinguishable intermediate stages 1 and 2 showed body volume and body colour one-third and two-thirds more advanced than zero, respectively. Mathematically speaking, stage 0 ranged from 0 to 1, stage 1 from 1.001 to 2, stage 2 from 2.001 to 3 and stage 3 from 3.001 to 4. N_0 therefore equals 4.

The digestion rate was measured in 2005 in Ngwarisha cave. Three refugia were chosen. All individuals in the first refugium were collected immediately. The other two refugia were gauze topped to prevent bugs inside the refugium from feeding and those outside the refugium from joining the refugium but leaving the bugs inside the refugium in their natural environment. The entire two refugia were collected 5 days and 10 days after the first one, respectively. The sex, developmental and digestion stage of all individuals were identified as described above. However, males and females were not available in a sufficient sample size and we only used nymphs to investigate the digestion rate. Our calculations on the feeding frequency shown below, therefore, rest on the (untested) assumption that adults have a similar digestion as nymphs.

Ten fully engorged (stage 3) and 10 starved (stage 0) last instar nymphs stored in ethanol were taken from our total sample. They were weighed to the nearest 0.01 mg (GR-202, A & D Instruments) and dried at 70 °C in an oven. Weight constancy was achieved after 24 h. The difference of the two means can be expected to represent the minimum dry weight of a full blood meal. Assuming a water content of 80% in the blood of *R. aegyptiacus* (based on humans and cattle – e.g., Lehane, 2005) we calculated how much blood was withdrawn by the entire bug population.

Statistical analysis was carried out using the statistical package S-PLUS (Insightful Corporation). We used generalised linear modelling in order to estimate which parameters had an effect on the digestion stage. Final models were those whose fit did not significantly improve upon addition or exclusion of additional parameters.

3. Results

The 20 bug refugia sampled had a surface area between 1.8 and 45.5 cm^2. They contained between nine and 298 bugs at 7.8 ± 3.6 (mean $\pm$ SD, range 2.1–14.4) bugs per cm^2. There was no correlation between population density and patch size ($r_s = 0.113$, $P = 0.636$, $n = 20$) indicating that a linear approximation can be used to estimate the number of bugs from the area of the refugium. The latter approach revealed that the number of bugs in a refugium can be predicted with 71% accuracy based on its area (Fig. 2). Thus estimated host and parasite population sizes, and therefore mean abundances, varied between caves but not so much between years in Makingeny (Table 1).

The mean digestion stages are summarised in Fig. 3 and Table 2. The digestion stage differed between males, females and nymphs and between caves (two-way analysis of variance (ANOVA), males, females, nymphs: $F = 6.58$, $df = 2$, $P = 0.0015$; cave $F = 23.63$, $df = 2$, $P \ll 0.0001$). However, males, females and nymphs differed from one another in the same way across all three caves (non-significant interaction: $F = 1.00$, $df = 4$, $P = 0.406$). The random samples of individuals moving about the cave had the lowest value for feeding status across all three caves (Fig. 3, asterisked samples). A statistical model based only on whether a sample was from a refugium or from individuals moving around, performed worse than a model using all refugia individually ($F = 3.194$, $P \ll 0.001$, samples nested in cave). This suggests that the variation between samples is not solely due to refugium or non-refugium type.

The average digestion status of nymphs decreased during food deprivation (Fig. 4). The median stage declined by 1 after 5 days and by 2 after 10 days. Therefore, the feeding status of nymphs declines by 1 approximately every 5 days, i.e., the decay rate b (see Eq. (2)) equals 0.2.

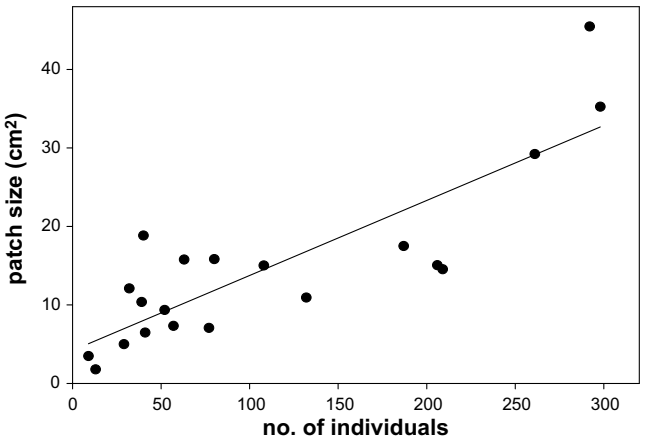

Fig. 2. The relationship between refugium size and the number of individuals in that refugium. The number of bugs *Afrocimex constrictus* can be predicted with an accuracy of 71% from the size of the refugium (R^2 of the regression is 0.71).

Table 1
Population size estimates of a fruit bat host and its parasite in three different caves

Cave/year	Host population size	Area of bug refugia (cm^2)	Bug population size (SD)	Mean abundance (SD)
Kitum 2004	15–20,000	3,200	24,960 (6720–46,080)	1.25 (0.33–2.3)
Makingeny 2004	100–120,000+	13,000	101,400 (27,300–187,200)	0.84 (0.22–1.56)
Makingeni 2005		17,749	138,400 (74,500–202,300)	1.15 (0.62–1.69)
Ngwarisha 2004	200,000+			
Ngwarisha 2005		384,858	3.002 mio bugs (1.62–4.39 mio)	15.01 (8.1–21.95)

mio, million.

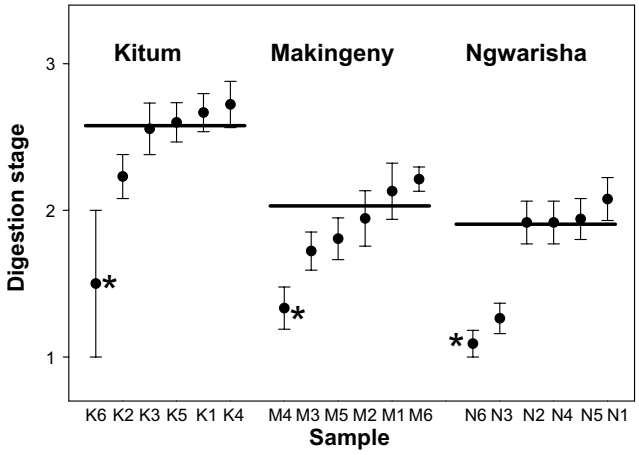

Fig. 3. The average digestion stage of *Afrocimex constrictus* individuals (ranging from 0 to 3) in six samples from each of three caves. Stage 0 refers to individuals that had not fed. Stage 3 refers to fully engorged individuals. The asterisked samples are individuals captured while moving across the cave walls or floors. All other samples are from refugia.

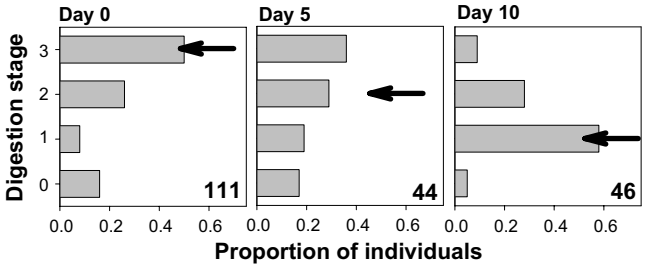

Fig. 4. The decline in digestion stage of *Afrocimex constrictus* nymphs over 10 days of food deprivation in situ in the field. The *x*-axis represents the proportion of individuals per digestion stage. The numbers in the right lower corner of each graph represent sample size. The black arrows denote the median digestion stage in each sample, indicating the digestion stage decreases on average 1 unit per 5 days.

Solving Eq. (2) for x produces a formula for calculating the average number of days since the last blood meal was taken

$$x = (N_0 - N(t))/b \quad (3)$$

Consequently we estimate that, on average, each individual of *A. constrictus* feeds every 7–10 days (Table 2).

The mean dry weight of fully engorged last instar nymphs was 3.85 ± 0.76 mg ($\pm$SD, $n = 10$), that of unfed last instar nymphs 1.18 ± 0.32 mg ($\pm$SD, $n = 10$). Again assuming that bugs engorge fully during a blood meal (as does the human bed bug *C. lectularius* in the laboratory), the difference between the fully engorged and unfed sample (2.67 mg) represents the dry weight of one meal. If so, and if dry weight comprises 20% of the blood of *R. aegyptiacus*, at last instar each *Afrocimex* withdraws about 13.3 mg blood, or 13.3 μL.

4. Discussion

We estimated host and parasite population sizes and calculated a mean parasite abundance of 0.84–15 bugs per bat host. As this estimate is not informative about the rate at which a host is contacted by a parasite we further calculated how frequently each bug, on average, feeds on a host. We also estimated how much blood is withdrawn per meal. All or some of our observations and calculations can be carried out in other haematophagous invertebrates. This will depend on the species' ecology. While our method of estimating the population size may be unsuitable for fleas and mosquitoes, our method of estimating the feeding frequency may be easy to use in studying fleas and mosquitoes. It may be possible to employ our approach to all blood sucking insects that can be randomly sampled and

Table 2
Digestion stage of nymphal, female and male *Afrocimex constrictus* in three different caves in the Mt. Elgon area, Kenya

Cave	Nymphs	Females	Males	Overall	Estd. feeding frequency ($days^{-1}$)
Ngwarisha	1.90 (67)	2.17 (40)	1.79 (52)	1.93	10.35
Kitum	2.62 (69)	2.36 (11)	2.53 (17)	2.58	7.1
Makingeny	2.19 (75)	2.00 (37)	1.88 (78)	2.02	9.9

Numbers in brackets are sample sizes. The digestion stages were classified from 0 (fully digested) to 3 (fully engorged). It is important to note that these classifications represent the stages 0–0.999, 1–1.999, 2–2.999 and 3–3.999. The feeding frequency can then be estimated from the difference of the maximum digestion stage to the mean digestion stage, divided by the digestion rate. See Section 3 for further details and Discussion for a critical consideration of the assumptions behind this rapid estimation method.

in which the feeding status can be quantified. For example, it has been suggested that human lice feed three times per day but this has not been demonstrated under natural conditions (Burgess, 1995).

The importance of other Cimicidae as pests to humans, livestock and wildlife arises because they cause blood loss, allergic reactions, disease transmission and secondary infections (Usinger, 1966; Marshall, 1981; Axtell, 1999; Brown and Brown, 1996; Reinhardt and Siva-Jothy, 2007). Several of these negative effects are directly related to parasite population size or feeding frequency and may, therefore, be estimated using our method, in particular in species regularly utilising human hosts such as *C. lectularius, Cimex hemipterus* and *Leptocimex boueti*. If infestations are so well established that distinct refugia have been formed, our way to estimate the parasite population sizes from refugia sizes may be adjusted for bed bugs and may allow a quantification of infestations of these emerging pests (Reinhardt and Siva-Jothy, 2007). For a range of taxa, our method can and should be used as a rapid and inexpensive pilot estimation for molecular studies that aim to elucidate a human biting specificity among individuals of a population (e.g., Michael et al., 2001; Mukabana et al., 2002).

The estimated feeding frequencies of once every 7–10 days may be higher than the actual rate if blood is naturally digested at a slower rate than assumed here. For example, we assumed that individuals from Kitum and Makingeny (which we did not measure) had the same digestion rate as the individual in Ngwarisha (which we did measure). However, it is possible that environmental differences between the caves lead to different digestion rates. It seems clear, though, that the differences are not caused by competition between bugs for food because in the latter case we had expected that the feeding frequency would be more similar between Kitum and Ngwarisha (which are similar in the mean parasite abundances) than between Makingeny and Ngwarisha as observed here. While relative humidity in the bug refugia was similar between Makingeny and Ngwarisha, the temperatures differed. In Makingeny, temperatures in the bug refugia were between 19 and 21 °C, in Ngwarisha between 18 and 24 °C (Reinhardt et al., unpublished data). If the lower temperature in Makingeny would lead to lower decay rates b of only 0.19 rather than 0.2 in Ngwarisha the feeding rate in Makingeny would be 10.4 and hence, very similar to Ngwarisha. This theoretical exercise highlights the importance of temperature in determining the decay parameter b. While we have no temperature readings from Kitum cave we may assume the proportion of adults to be an indicator of temperature-related developmental speed of the population. Thus, Kitum (12.3% adults) can be assumed to be colder than Makingeny (16.6% adults) and Ngwarisha (27.8% adults). The decay rate would subsequently be slower and indeed the estimated feeding frequency would be slower. Additional variation may come from inter-individual differences in the decay rate b: some nymphs (Fig. 2) and females (unpublished data) were still fully engorged after 10 days of food deprivation.

The estimations for feeding rates might also fluctuate if, during the shorter nymphal development, (i) younger nymphs obtained smaller but more frequent meals and (ii) the digestion rate in our food-deprived samples was unnaturally low. The latter might have occurred if our confinement of the bugs to the refugium had lead to reduced movement and therefore reduced metabolism and digestion rate. The digestion rate of free-ranging cimicids can probably only be addressed by mark-recapture studies in which the digestion stage (or the body weight) is repeatedly recorded.

Our estimate of the feeding frequency (but not the total blood loss) would be too low if *A. constrictus* does not fully engorge at each blood meal but feeds more frequently. While frequently approaching the host is potentailly risky for any temporary ectoparasite, full engorgement may be associated with increased costs of blood digestion or storage (Krasnov et al., 2003; Sarfati et al., 2005 for fleas), with decreased motility on the host (Lehane, 2005) or, in the case of adult cimicids, with an increased mortality rate due to superfluous matings (Stutt and Siva-Jothy, 2001; reviewed Siva-Jothy, 2006; Reinhardt and Siva-Jothy, 2007). In *A. constrictus* it may be costly to both sexes because males mate with other males (Carayon, 1966).

Finally, the decay may be exponential rather than linear (see Eq. (1)). This would result in an underestimated feeding rate in recently fed individuals but an overestimation in unfed individuals. Since the cross-section we collected in the field indicated that most sites showed intermediate values of mean digestion status (Fig. 3) we believe that a linear approximation is justified.

Considering the above factors which might influence the digestion rate does not determine whether we were more likely to over- or under-estimate feeding frequency. Processes leading to either are quite possible and may occur simultaneously in which case the two sources of error would partially negate each other.

The estimated blood loss depends largely on the accuracy by which mean parasite abundance, feeding frequency and meal size can be estimated. Our estimated mean abundances of 0.84–15.01 bugs per host would result in 11.2–199.6 μL of blood being withdrawn from each bat approximately every 7–10 days, i.e., averages of 1.12–28.5 μL per day. Whether such blood loss (perhaps associated with anaemic symptoms as observed in one human volunteer – Usinger, 1966) translates into reproductive disadvantages for bats, and therefore has evolutionary consequences is unknown as, in fact, for any cimicid-bat relationship (Reinhardt and Siva-Jothy, 2007).

Cimicids are vectors for several diseases (e.g., Usinger, 1966; Brown et al., 2001; Reinhardt and Siva-Jothy, 2007) albeit not for humans. In addition to the mean abundance of the parasite, the parasite population size has itself been shown to be an important predictor of the rate of virus transmission in a cimicid bug (Brown et al., 2001).

Such insect-borne viruses and the resulting wildlife diseases may be relevant for our study system because fruit bats not only harbour viruses harmful to themselves (such as the Lagos virus – Merdith and Standing, 1981; Markotter et al., 2006) but also some that can be fatal to humans when humans come into contact with fruit bats, eg the Nipah (Butler, 2004) and Ebola viruses (Leroy et al., 2005) and, in the case of the caves studied here, Marburg Haemorrhagic Fever Virus (Bausch et al., 2006).

Our method of calculating the feeding frequency is simple and therefore very suitable for the field. However, much better estimates can be obtained when N is replaced by more precise measurements, such as the body weight decline. If the decline is sufficiently frequently measured it is possible to analyse whether the decay rates are exponential or linear. Likewise, the decay rates can be separately calculated for each instar as well as separately for males and females. In the present study, we only had a sample size for nymphs that was large enough to estimate the digestion rate and we assume that males and females are similar to nyphs in this respect.

Lehane (2005) summarises available data on the digestion rate and the meal size for several fully engorged blood-sucking insects. We suggest that the quantification of the digestion stage in random field samples of these insects may provide good estimates of their average feeding frequency in the field.

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