Habitat fragmentation and haemoparasites in the common fruit bat, *Artibeus jamaicensis* (Phyllostomidae) in a tropical lowland forest in Panamá

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SUMMARY

Anthropogenic influence on ecosystems, such as habitat fragmentation, impacts species diversity and interactions. There is growing evidence that degradation of habitats favours disease and hence affects ecosystem health. The prevalence of haemoparasites in the Common Fruit Bat (*Artibeus jamaicensis*) in a tropical lowland forest in Panamá was studied. We assessed the relation of haemoparasite to the general condition of the animals and tested for possible association of haemoparasite prevalence to habitat fragmentation, with special focus on trypanosomes. Overall, a total of 250 *A. jamaicensis* sampled from fragmented sites, here man-made, forested islands in Lake Gatùn, and sites in the adjacent, continuous forest in and around the Barro Colorado Nature Monument were examined. Using microscopy and DNA-sequencing 2 dominant types of haemoparasite infections, trypanosomes and *Litomosoides* (Nematoda) were identified. Trypanosome prevalence was significantly higher in bats from forest fragments, than in bats captured in continuous forest. We attribute this to the loss of species richness in forest fragments and specific characteristics of the fragments favouring trypanosome transmission, in particular changes in vegetation cover. Interestingly, the effect of habitat fragmentation on the prevalence of trypanosomes as multi-host parasites could not be observed in *Litomosoides* which probably has a higher host specificity and might be affected less by overall diversity loss.

Key words: *Artibeus jamaicensis*, habitat fragmentation, diversity, multi-host parasite, haemoparasites, *Trypanosoma*, *Litomosoides*.

INTRODUCTION

Habitat fragmentation and degradation result in drastic changes to the biological and physical environment, which in turn impact species richness as well as ecosystem functioning and services (Sih *et al*. 2000; Diamond, 2001; Allan *et al*. 2003; McCallum and Dobson, 2002; Fahrig, 2003; Patz *et al*. 2004; Tabarelli *et al*. 2004). This is particularly true for tropical regions (Laurance *et al*. 2002; Wade *et al*. 2003). As a general trend, fragmentation reduces species richness and abundance with only few adaptable species that might increase in numbers (Cosson *et al*. 1999; Terborgh *et al*. 2001; Meyer and Kalko, 2008*a*).

Habitat fragmentation is increasingly recognized as a crucial factor that impacts on the health and fitness of the animals left in the fragments (Martínez-Mota *et al*. 2007). Fragmentation and reduction in species richness have been connected with an increase in diseases and multi-host parasitism (Allan *et al*. 2003; Gillespie *et al*. 2005; Chapman *et al*. 2005; Gillespie and Chapman, 2006, 2007; Keesing *et al*. 2006; Salzer *et al*. 2007). A better understanding of the links between fragmentation, parasitism, and the resulting physiological reactions of the affected animals is necessary in order to predict and mitigate current and future consequences of habitat fragmentation on the fitness of wildlife.

Animals with reduced fitness are likely to be less efficient at providing crucial ecosystem services, such as plant recruitment through seed dispersal and pollination or control of pest insects. In addition, they are more prone to diseases and may also act as reservoirs for human-pathogenic, zoonotic diseases (Chapman *et al*. 2005).

Bats in tropical lowlands are excellent indicators of ecosystem health and ecosystem change (Medellin *et al*. 2000; Castro-Luna *et al*. 2007; Willig *et al*. 2007) due to their species richness, differential reactions to fragmentation and crucial ecological functions (Patterson *et al*. 2003). We studied the impact of habitat fragmentation on the health of bats in the Barro Colorado Nature Monument (BCNM)

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in Panamá. There, the flooding of the Panama Canal created an environment with a mosaic of islands varying in size and degree of isolation.

With a 2-year comprehensive data set on patterns of bat species richness on the islands and the mainland as a basis, we selected the common fruit bat, *Artibeus jamaicensis*, as a study organism to assess potential links between parasitism, health, and fragmentation. We focused on *A. jamaicensis* because of its high abundance, its well-known ecology (Handley *et al*. 1991) and its distinct reaction to habitat fragmentation (Meyer and Kalko, 2008*b*). Although highly mobile on the population level (Handley *et al*. 1991), *A. jamaicensis* capture-recapture studies in the BCNM and nearby islands demonstrated rather localized and consistent habitat use on the individual level (Handley *et al*. 1991; Meyer and Kalko, 2008*b*) suggesting a rather distinct subpopulation structure.

Of all the parasites of bats, studies on trypanosomes are of particular interest due to their morphological similarity with *Trypanosoma cruzi cruzi*, the aetiological agent of Chagas' disease in man (Hoare, 1972; Marinkelle, 1976; Bower and Woo, 1981; Molyneux, 1991; Steindel *et al*. 1998; Grisard *et al*. 2003). Trypanosomes have long been known as widespread parasites of bats (Hoare, 1972) and are considered to be generalists, occurring in most bat families (Molyneux, 1991). Although little is known about the transmission of trypanosomes in bats, arthropods, especially assassin bugs, have been discussed as the main vectors (Molyneux, 1991).

Ever since bats have been identified as possible reservoirs of *Trypanosoma cruzi* (Sousa, 1972), they have been included in medical epidemiological surveys. This interest has been fuelled by the presence of bat-specific trypanosomes, which do not differ morphologically from *T. cruzi cruzi* (Hoare, 1972; Marinkelle, 1976; Stevens and Brisse, 2004). In previous decades large efforts researching the possibility that bats could be reservoirs for human pathogenic haemoparasites have been undertaken (Hoare, 1972; Sousa, 1972; Marinkelle, 1982). However, none of those studies took into account the bat's physiology and ecology or the role of the environment. This knowledge, however, is essential for adequate risk assessments about chances of disease transmission, should it be infectious and transmittable between bats and humans.

The incidence of infectious diseases, especially general vector-borne parasitism, often increases as species diversity decreases. This has already been shown for the tick-borne Lyme disease caused by *Borrelia burgdorferi* with its associated rodent hosts (Ostfeld and Keesing, 2000*a*, *b*). Similar findings originate from studies on American trypanosomiasis in Brazil (Vaz *et al*. 2007) and in Colombia where human infection occurred less frequently in areas with a high diversity of mammal species than in areas harbouring only a few species (Travi *et al*. 1994). Based upon these findings and the fact that parasitism generally tends to increase in disturbed habitats (Walsh *et al*. 1993; Patz *et al*. 2000; Lafferty and Holt, 2003; Chapman *et al*. 2005) we hypothesized that habitat fragmentation in the BCNM should result in an increase in trypanosome prevalence in bats with higher prevalence in individuals inhabiting small habitat fragments. The effects of habitat disturbance on health has already been identified as a crucial factor in this area in the early 1900s where the construction of the Panama Canal catalysed a massive yellow fever outbreak, a vector-borne viral disease that occurred at that time in howler monkeys (*Alouatta palliata*) (Cook *et al*. 2004).

The main goal of our study was to determine haemoparasite prevalence of the common fruit-eating bat, *A. jamaicensis*, with special emphasis on trypanosomes, and to link it to the bat's physiology focussing on differential blood counts, weight and body length, and to environmental factors, particularly on local bat diversity.

MATERIALS AND METHODS

Study species and sites

We studied haemoparasites of the common fruit bat, *Artibeus jamaicensis* (Phyllostomidae; Leach 1821), a large bat with a predominantly frugivorous diet (Reid, 1997) and high abundance at our study site (Gardner *et al*. 1991; Kalko *et al*. 1996). The main part of the study was conducted in the 5400 ha Barro Colorado Nature Monument (BCNM) near the Panama Canal in Lake Gatún, Republic of Panamá, in the rainy season from 19 September to 16 November 2005. The BCNM area was fragmented into a variety of islands and peninsulas by the damming of Lake Gatún for the construction of the Panamá Canal in 1914 (Leigh, 1999). We compared haemoparasites from bats that were caught on 4 islands differing in size (2·5–50 ha) and degree of isolation (155–2247 m) from 3 sites in the continuous rainforest of Soberania National Park (~22·000 ha) on the mainland (Fig. 1).

Data collection

Data collection followed the Institutional Animal Care & Use Committee (IACUC) protocol approved by the Smithsonian Tropical Research Institute (STRI) and the research permits issued to the principal investigators. Each site was sampled on one night for about 12 h from dusk until dawn. The bats were caught in 6 ground nets (6 m by 2·5 m; mesh size 35 mm; 4 shelves) and a canopy net when catching in cooperation with C. Meyer (for details see Meyer and Kalko, 2008*b*). The ground nets accounted for about 69 mist net hours per site (1 mist

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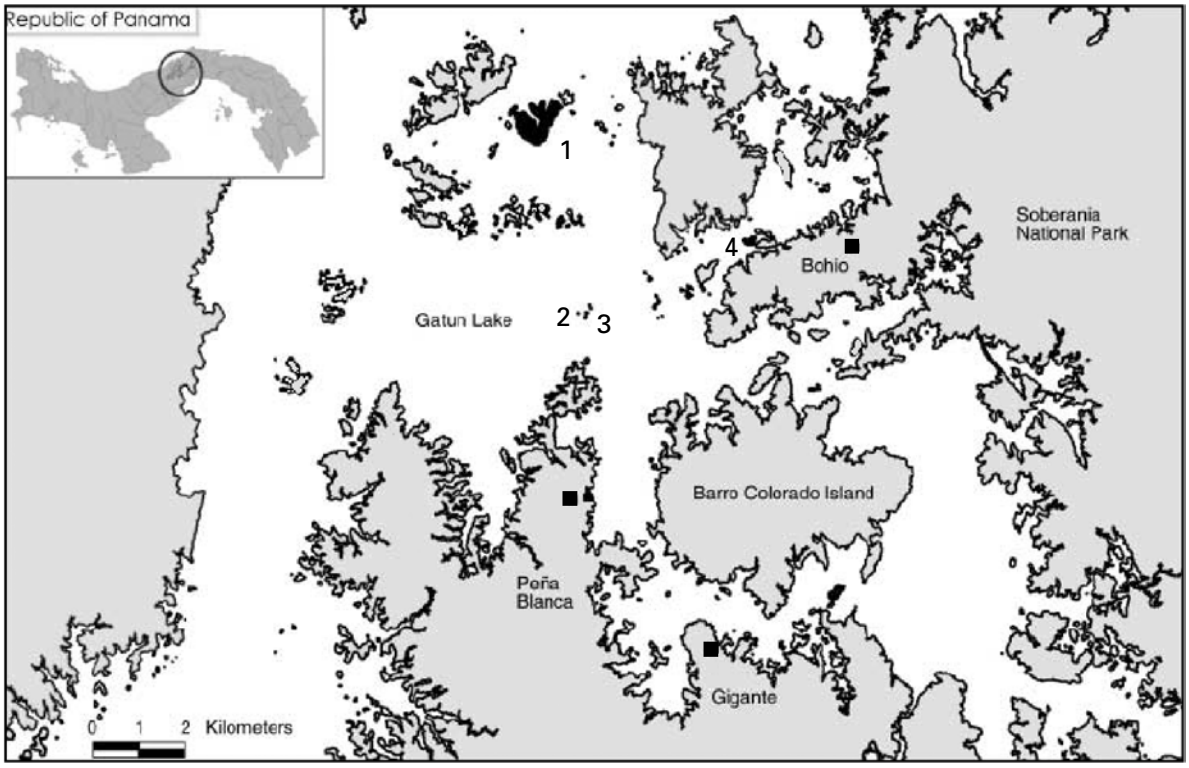

Fig. 1. Capture sites in the BCNM in central Panama (inset) modified from Meyer *et al*. (2008). Highlighted in black are the locations of the 4 islands (numbered) and the 3 mainland sites in continuous forest (■).

net hour = one 6 m mist net open for 1 h) and the canopy net for about 38 mist net hours per site. Nets were checked regularly at least every 20 min. Entangled bats were taken out immediately upon discovery, temporarily put into a soft cloth bag and taken to a temporary field camp for processing. We recorded the following data using a standard protocol: location, date, and time of capture, net location, forearm length (to the nearest 1 mm with a calliper), weight (to the nearest 0·1 g with a Pesola spring balance), gender, age class (juvenile, subadult and adult according to the ossification of the phalanges), and reproductive status (pregnant, lactating, post-lactating, and non-reproductive in females; reproductive active and inactive in males). To recognize recaptures, all subadult and adult bats were marked with a numbered aluminium ring on a stainless-steel necklace that was fitted individually. Both procedures followed the protocols of Handley *et al*. (1991). Juveniles received a waterproof colour marking with a pen (Edding, Ahrensburg, Germany) on their back that lasted approximately 24 h. Recaptures were released at the study site without re-sampling. Blood samples were taken from the cephalic vein. The vein was punctured with a 27 G needle (Becton Dickinson, Heidelberg, Germany). Blood was taken up with an unheparinized 75 μl capillary (Brand, Wertheim, Germany) and immediately transferred to a collection tube (Eppendorf, Hamburg, Germany) containing 2 μl 0·1 M EDTA (Merck, Darmstadt, Germany). Up to 150 μl of blood were collected and the EDTA concentration was adjusted to give a final concentration of 0·02 M. Afterwards, the bats were fed sugar water and released at the capture site.

Thin blood smears were made within 3 h of blood taking, following the method described by Brown (1993) and Houwen (2000), using the cover-slip technique. A vol. of 2·5 μl EDTA-blood was used per blood smear, each on a separate microscope slide (Marienfeld, Lauda-Koenigsfeld, Germany). Several blood smears per bat were made. Blood smears were dried and fixed in pure methanol for 5 min, and again for 10 min shortly before staining.

The blood slides were stained with the May-Grünwald-Giemsa stain and examined microscopically as described by Marinkelle (1982) and Sehgal *et al*. (2001). Screening was done at 630 × magnification and classification of potential parasites with 1000 ×. Each smear was checked from 20 min to 2 h covering a standardized area of 2·5 μl blood. Blood parasites were documented and measured to the nearest 0·01 μm with the Axiovision 3·1 programme (Zeiss, Jena, Germany). For morphological identification, parasite length, width, and specific parameters such as nucleus and kinetoplast size and position were measured following the method of Hoare (1972). The parasite load was calculated from the number of parasites in the previously defined quantity of blood (2·5 μl). Direct microscopy

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is a standard method for trypanosome detection in the acute phase of the infection. It is rare to find the parasites after the 6th week (Palmer and Reeder, 2001). The differential blood count (DBC) was adapted from Brown (1993) using the May-Grünwald-Giemsa stained blood smears. A total of 300 white blood cells per blood smear were investigated.

Trypanosome DNA analysis

From 17 infected bats a larger amount of blood (>40 μl) could be taken for genetic analysis. Blood samples were treated and lysed in 500 μl of lysis buffer (10 mM Tris-HCl pH 8·0, 100 mM EDTA, 2% SDS) according to Sehgal *et al.* (2001). Nuclear DNA was extracted from the lysed blood samples with the QIAamp Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, modified by an initial 15 s incubation with proteinase K (Qiagen, Hilden, Germany).

The ssrRNA gene sequences were amplified following the method of Noyes *et al.* (1999) with a nested PCR assay amplifying a portion of the 16S rRNA gene using external primer TRY927F (GAAACA AGAAACACGGGAG) and TRY927R (CTACTGGGCAGCTTGGA) and internal primer SSU561F (TGGGATAACAAAGGAGCA) and SSU561R (CTGAGACTGTAACCTCAA AGC).

For PCR, 1 μl from the DNA extraction eluate was added to a final volume of 25 μl in the presence of 1·5 mM Mg^{2+}, 1 mM dNTPs, 1× PCR Puffer Qiagen, 0·25 μl Qiagen Taq, 2 mM each primer for 35 cycles of 60 s at 90 °C, 90 s at 55 °C, 90 s at 72 °C, after a hot start at 95 °C for 10 min; followed afterwards by 10 min at 72 °C. Then 0·5 μl of undiluted product of the first round reaction using primers TRY816F/R was used as template in the second round under the same conditions with primers SSU450F/R. The product then was loaded onto a 2% agarose gel, run and viewed under UV light to confirm amplification. To obtain DNA for sequencing, 1 μl of the first round PCR was used in a 100 μl reaction mixture with the primers SSU651F/R (see above). Cycling conditions were the same as for the PCR above. PCR products were then run on a gel (conditions see above) and the amplicon bands were extracted. In the case of there being more than 1 band in the gel, each band was extracted separately. Primers and dNTPs were removed on a QiaQuick DNA purification column (Qiagen, Hilden, Germany). After precipitation with ethanol the PCR products were sequenced using an ABI 310 DNA sequencer (Applied Biosystems, Darmstadt, Germany) and an ABI PRISM BigDye Terminator Cycle Sequencing Kit according to the manufacturer's instructions. Sequence analysis was performed by a Basic Logical Alignment Search Tool algorithm (BLAST) search (http://www.ncbi.nlm.nih).

Statistics

Statistical analyses were performed with STATISTICA 6.1 (StatSoft, Tulsa, OK, USA) and Sigma Stat 3.11.0 (Systat Software, Richmond, CA, USA). Data were tested for normality and homogeneity of variance. Frequencies were tested with the Chi-square test (Yates correction for 2×2 tests; larger 2-dimensional contingency tables were performed according to Precht (1982)). Data sets that did not fit a normal distribution or had a small sample size ($N < 25$ per group) were compared with non-parametric tests, in particular the Mann-Whitney-U-test for comparison of 2 groups, Kruskall-Wallis-Anova for comparison of more than 2 groups, and Spearmann's correlation on ranks. The level of significance was set to $\alpha = 0{\cdot}05$. Dunn's method served as post-hoc test for comparisons between more than 2 groups. We applied Pearson product-moment correlation to normally distributed data sets (site data, cell, and parasite measurements). To exclude random effects of significance, the false discovery rate control was applied (FDR; Verhoeven *et al.* 2005).

RESULTS

Haemoparasite detection and morphology

Blood samples from 257 individual bats ($N = 91$ on the mainland and $N = 145$ on the islands) resulted in blood smears from 23–45 individuals per site (Table 1). We found 2 types of haemoparasites in the bats' blood, namely *Trypanosoma cruzi*-like kinetoplastids (Protozoa) and sheathed worms (Nematoda) of the genus *Litomosoides*. The trypanosomes in the blood of *A. jamaicensis* resemble the *T. cruzi*-like type and belong to the subgenus *Schizotrypanum* Chagas, 1909 (Hoare, 1972). Morphologically, most trypanosomes had a C- or S-shaped form (Fig. 2A). As is characteristic for trypanosomes of the subgenus *Schizotrypanum* there were long, slender-formed trypanosomes with an elongated nucleus and broad, short forms with an oval-round nucleus. They averaged 18·5 μm in length including the freestanding flagellum (range: 15·3–21·5 μm; $N = 34$). There were no morphological differences between the *T. cruzi cruzi* type and the two *T. cruzi marinkellei* types. The nucleus was oval or elongated, with a mean length of 2·7 μm and a mean width of 2·0 μm. The nuclear index was 1·4–1·7. Sequence analysis of an ssrRNA gene fragment (Noyes *et al.* 1999) from blood samples from 17 infected bats indicated that all of these bats were infected by 3 variants of the *T. cruzi*-complex. One variant, occurring in 8 bats, had a 95% homology to the *Trypanosoma cruzi* strain MT3663, AF288660·1 (amongst other *T. cruzi cruzi* strains) in the NCBI databank. Furthermore, we detected 2 sequences that were very similar (98%) to the *T. cruzi marinkellei*, isolate B7, AJ009150·1 sequence

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Table 1. Capture sites and parasite incidences

(Data on species richness and relative abundance of *A. jamaicensis* were provided by Meyer *et al.* (2008). Relative abundance was measured in bats captured per mist net (2·6 m × 6 m) and hour.)

Site	Fragmentation	No. of bat species	Relative abundance (*A. jamaicensis*)	*Trypanosoma* prevalence (%)	*Litomosoides* prevalence (%)	*N* (Total)
Guanabano	Island	5	1·14	26·7	33·3	45
Guava	Island	7	1·07	15·0	7·5	40
Cacao	Island	12	0·29	8·3	8·3	24
Leon	Island	13	0·79	5·6	5·6	36
Pena Blanca	Mainland	23	0·23	6·9	21·0	43
Bohio	Mainland	22	0·40	3·0	18·2	33
Gigante	Mainland	23	0·15	0·0	4·4	23

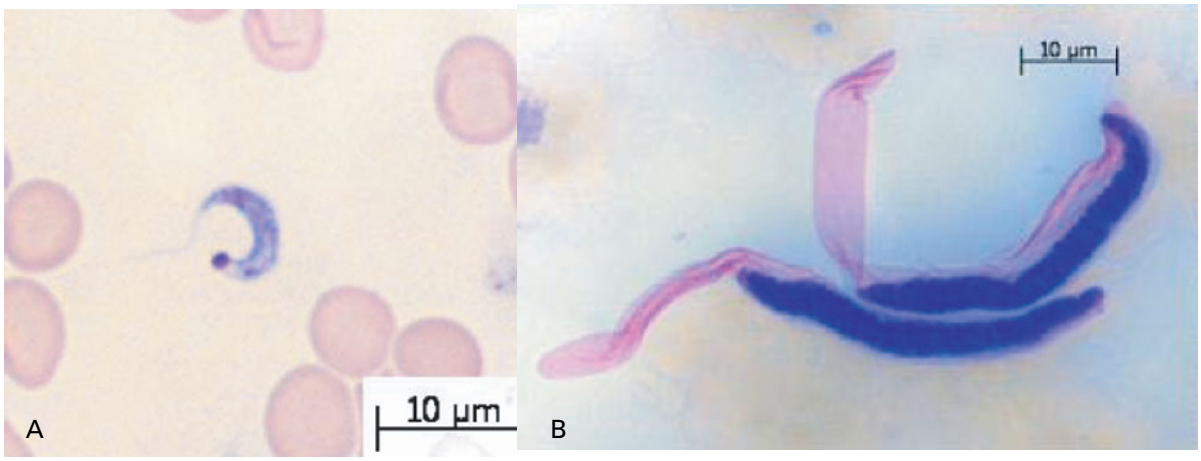

Fig. 2. (A) *Trypanosoma cruzi*-like trypanosome from *A. jamaicensis*. (B) *Litomosoides sp.* microfilariae from *A. jamaicensis*.

in the NCBI databank. One occurred in 10 bats, the other in 2 bats. We also found 1 sequence, identified as 100% *T. rangeli*, isolate G5, EF071582·1 in 1 of the bats. There were 5 double infections as indicated by 2 bands in the agarose gel and subsequently 2 different sequences being obtained. *Trypanosoma rangeli*, which was present in 1 bat as a co-infection, could not be seen in the blood smears.

The bats' blood also featured sheathed microfilariae with an average length of 56·2 μm ($N=37$) varying from 40·4 to 88·5 μm and an average width of 4·1 μm and a range of 3·3–4·8 μm. The head was attenuated and the nuclei were very close together. The outstanding feature of these microfilariae was their sheath, which showed semi-transparent pink in the stain (Fig. 2B). The microfilariae were identified by O. Bain (Parasitologie comparée, Muséum National d'Histoire Naturelle, Paris) as belonging to *Litomosoides* Chandler, 1931, Onchocercidae (Nematoda: Filarioidea). In the present samples, 2 types of microfilariae were distinguished, indicating the presence of 2 species of *Litomosoides*. It is not possible to accurately identify the species without checking the adult worms, which were not available to us.

Haemoparasite prevalence and load

Of the 255 *A. jamaicensis* checked microscopically, 77·6% of the animals ($N=198$) had no haemoparasites. Active trypanosome infection was found in the blood of 26 individuals (10·2%) and active infection by *Litomosoides* was detected in 40 individuals (15·7%).

The loads of trypanosomes and sheathed worms varied widely. At a detection limit of 400 parasites per ml (maximum sensitivity was 1 parasite per slide), the trypanosome load ranged from 0 to 758462/ml with a mean of 4725/ml and a standard deviation of 48750/ml. The load of *Litomosoides* was lower, with a range from 0 to 278409/ml and a mean of 3681 ± 25348/ml. A small fraction of bats from various islands ($N=9$; 3·5%) revealed a double infection with both haemoparasites. Bats infected with 1 type of parasite were significantly more likely to have the other parasite as well (Chi-square;

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Table 2. DBC of bats with no detected haemoparasites, bats found with trypanosomes, bats with *Litomosoides* and bats with both types of haemoparasites

	Bats with no detected haemoparasites ($N=25$)	Bats with Trypanosomes ($N=17$)	Bats with *Litomosoides* ($N=21$)	Bats with both trypanosomes and *Litomosoides* ($N=9$)
Juvenile granulocytes (%)	4·0	6·3	5·8	6·2
(*Standard dev.*) (%)	*1·7*	*2·6*	*1·9*	*2·3*
Mature granulocytes (%)	59·4	48·7	56·9	50·7
(*Standard dev.*) (%)	*10·5*	*17·1*	*12·2*	*17·4*
Eosinophils (%)	1·2	1·4	2·2	3·0
(*Standard dev.*) (%)	*0·6*	*0·7*	*1·1*	*1·5*
Basophils (%)	0·8	0·9	0·9	0·9
(*Standard dev.*) (%)	*0·4*	*0·2*	*0·7*	*0·4*
Monocytes (%)	5·4	6·0	6·1	6·0
(*Standard dev.*) (%)	*3·0*	*3·0*	*3·2*	*2·2*
Lymphocytes (%)	29·2	36·6	28·0	33·1
(*Standard dev.*) (%)	*9·4*	*15·6*	*10·2*	*17·2*

$P=0{\cdot}0051$; Yates Correction $P=0{\cdot}011$). However, there was no significant correlation between the loads of the two parasites (Product-moment correlation; $r=0{\cdot}35$, $P>0{\cdot}05$).

Haemoparasites and bat physiology

Vital parameters in *Artibeus jamaicensis*, in particular body mass and body size (represented by forearm length; Handley *et al*. 1991), did not differ significantly between uninfected individuals and bats with haemoparasites. There were no gender-related trends concerning parasite prevalence. Overall, neither the trypanosome nor *Litomosoides* load of adult bats differed with respect to reproductive state.

Differential blood count (DBC)

The relative number of the different types of white blood cells was established for 69 *A. jamaicensis*. The DBC of the bats with no haemoparasites detected ($N=22$) was comparable to the DBC of healthy humans (data not shown; Lüllmann-Rauch, 2003). The DBC was also made for more than half of the individuals that were infected with sheathed worms ($N=21$), for all bats with trypanosomes but without sheathed worms ($N=17$) and for all bats that were infected by both trypanosomes and sheathed worms ($N=9$). There were several differences and trends concerning the percentages of neutrophilic and eosinophilic granulocytes and lymphocytes (see Table 2) in infected bats compared with non-infected bats. A significant left shift was found in infected bats (Kruskal-Wallis-Test: H (3, $N=69$) $=16{\cdot}36$, $P=0{\cdot}0010$; Posthoc: Dunn's method, $P<0{\cdot}05$; Fig. 3).

Eosinophilic granulocytes had a highly significant, distinct distribution in bats with parasites (Kruskal-Wallis-Test: H (3, $N=69$) $=18{\cdot}68$, $P=0{\cdot}00003$; Posthoc: Dunn's method; $P<0{\cdot}05$) where individuals infected with *Litomosoides* had a significantly higher number of eosinophils in contrast to bats without *Litomosoides*-infection (categories: no haemoparasites, trypanosomes only). They were characterized by low percentages of eosinophilic granulocytes as is normal for bats without parasites. We did not find a significant difference in the relative density of eosinophilic granulocytes between bats that were only infected with sheathed worms and bats that were infected with sheathed worms and trypanosomes. The number of lymphocytes tended to be higher in individuals infected with trypanosomes.

Parasites and habitat fragmentation

On the islands, trypanosome prevalence in bats (13·9%) was significantly higher than on the mainland sites with an average prevalence of 4·3% (Chi-square $=7{\cdot}95$; D.F. $=1$; Yates $P=0{\cdot}0091$). *Litomosoides*, however, were evenly distributed among islands (13·7%) and mainland (16·1%) (Chi-square $=0{\cdot}02$; D.F. $=1$; Yates $P=0{\cdot}97$). However, further analysis revealed a strong variation of trypanosome prevalence between the islands. That is why an interpretation of the specific situation of each island and a further comparison between the islands was necessary.

Trypanosome prevalence differed strongly between sites (Chi-Square $=19{\cdot}40$; D.F. $=6$; $P<0{\cdot}01$). On the far island Guanabano, with a high impact of fragmentation (Meyer and Kalko, 2008*b*), trypanosome prevalence in *A. jamaicensis* was highest with 26·7%. On Guava, the other far island with a high impact of fragmentation (Meyer and Kalko, 2008*b*), 15% of *A. jamaicensis* were infected with

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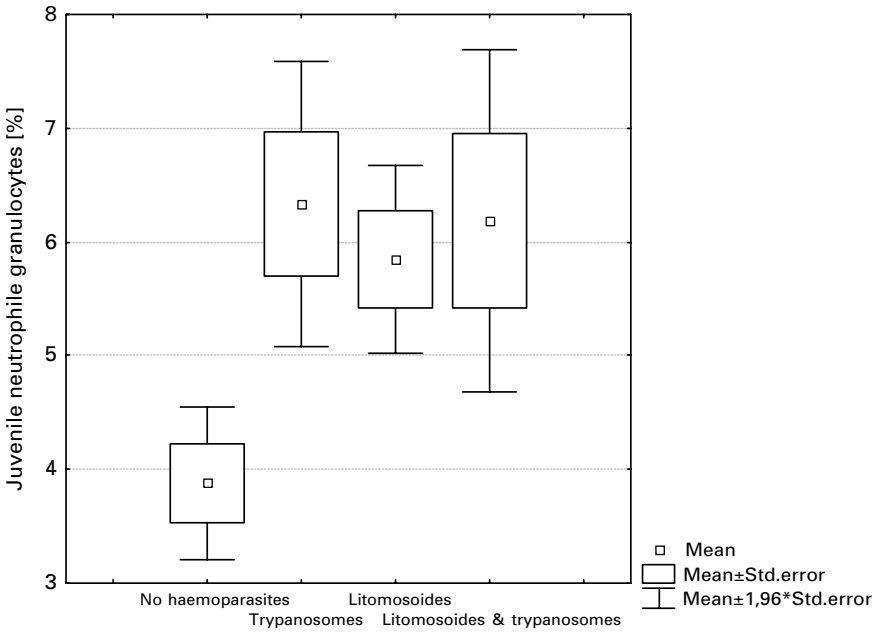

Fig. 3. Percentage of juvenile neutrophilic granulocytes in *A. jamaicensis* with and without parasites. Bats infected with trypanosomes or *Litomosoides*, or both, had significantly more juvenile neutrophilic granulocytes than uninfected individuals ($P=0{\cdot}001$).

trypanosomes. The two less fragmentation-affected islands, Cacao and Leon (Meyer and Kalko, 2008*a*, *b*), had significantly lower trypanosome prevalence (8·3% and 5·6% respectively). Trypanosome prevalence in *A. jamaicensis* was below 7% on the mainland sites. On Gigante, 1 of the 3 mainland sites, none of the *A. jamaicensis* ($N=23$) had any trypanosomes.

Whereas *Litomosoides* were also unevenly distributed among the sites (Chi-Square = 19·80; D.F. = 6; $P<0{\cdot}01$), with a prevalence ranging from 4·4% to 33·3%, it did not follow the same pattern as the trypanosome prevalence. In contrast to trypanosomes, infections with sheathed worms were found at all sites. Similar to the trypanosomes, the highest prevalence (33·3%) was on the island Guanabano. However, this site was followed by the mainland sites Pena Blanca and Bohio with a prevalence of approximately 20%. The other locations had *Litomosoides* prevalence ranging from 4·4% to 8·3% (Table 1).

The islands differ in the degree of isolation measured in bat species richness loss compared to the mainland (Table 2). For more details on the distribution of bats species and relative abundance see Meyer and Kalko (2008*a*, *b*). The far islands Guanabano and Guava, both with high trypanosome prevalence, were characterized by a high relative abundance of *A. jamaicensis* (measured in number of individual *A. jamaicensis* caught per mist net and hour) and a low overall number of other bat species and their abundance. In contrast, bat species richness was higher and relative abundance of *A. jamaicensis* lower on the islands Cacao and Leon, where the prevalence of trypanosomes was intermediate. On the mainland sites, overall bat species diversity was highest, whereas relative abundance of *A. jamaicensis* and trypanosome prevalence were both lower than on the islands.

The percentage of *A. jamaicensis* with trypanosomes was significantly related with bat species richness (Linear regression; Trypanosome prevalence = 24·0–0·98 × number of bat species; $R^2=0{\cdot}71$; $P<0{\cdot}02$; Fig. 4). The lower the total number of bat species at a site, the higher the percentage of *A. jamaicensis* infected with trypanosomes. We also found a significant correlation between trypanosome prevalence and relative abundance. Here, higher relative abundance of *A. jamaicensis* was related to a higher prevalence of trypanosomes (Linear regression; Trypanosome prevalence = −0·8210 + 17·527 × relative abundance of *A. jamaicensis* (in bats per mist net hour); $R=0{\cdot}82$, $R^2=0{\cdot}66$; $P<0{\cdot}03$; Fig. 5).

There was no significant correlation between the number of *A. jamaicensis* infected with *Litomosoides* and the number of bat species or the relative abundance of *A. jamaicensis* present at each site.

DISCUSSION

The main objective of our preliminary study was to investigate the haemoparasites of the neotropical fruit bat *Artibeus jamaicensis* within an anthropogenically fragmented landscape in Panama. We found 2 main types of haemoparasite, trypanosomes of the *T. cruzi* complex and sheathed worms (Nematoda). The prevalence of the trypanosomes

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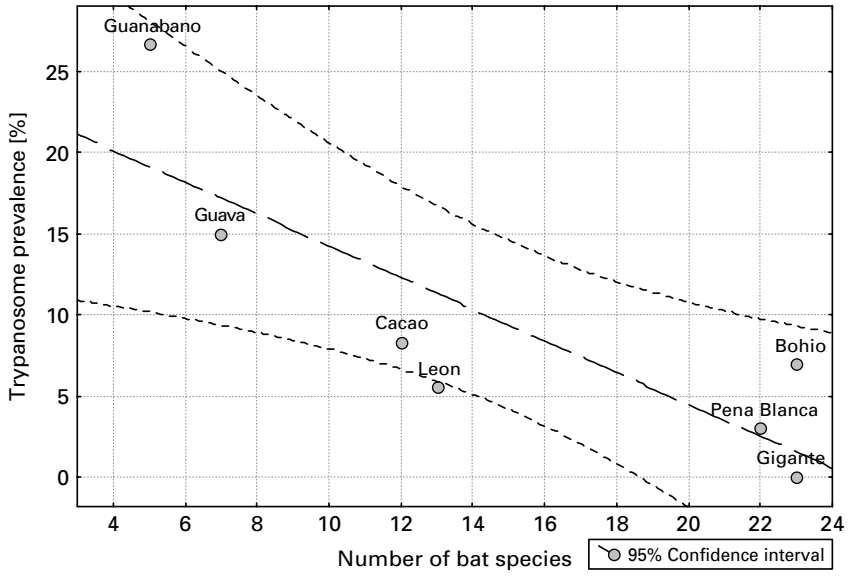

Fig. 4. Correlation between prevalence of trypanosomes in *A. jamaicensis* and bat species richness at different locations. Scatter plot: Species richness *vs* Trypanosome prevalence (MD case by case). Trypanosome prevalence = 24·017 − 0·9767 × Number of bat species. Correlation: r = −0·8386. The relationship is significant with $P < 0·02$.

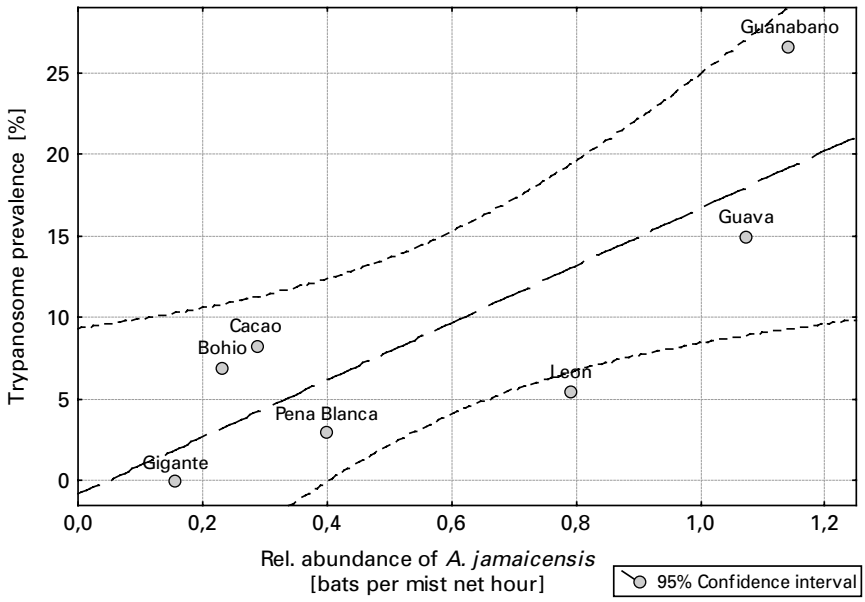

Fig. 5. Correlation between prevalence of trypanosomes in *A. jamaicensis* and relative abundance of *A. jamaicensis* at different locations. Scatter plot: Relative abundance of *A. jamaicensis vs* Trypanosome prevalence (MD case by case). Trypanosome prevalence = −0·8210 + 17·527 × Relative abundance of *A. jamaicensis*. Correlation: r = 0·80883. The relationship is significant with $P < 0·02$.

was associated with bat species richness and the density of *A. jamaicensis*, which in turn are linked to habitat fragmentation (Meyer and Kalko, 2008*b*).

The trypanosomes morphologically strongly resembled *T. cruzi* (*sensu latu*) in size, kinetoplast and nuclear index (nuclear index *T. cruzi* 0·9–1·7; *T. vespertilionis* 2·4–3·3; Hoare, 1972). Molecular identification of a trypanosome ssrRNA gene sequence from 17 of the infected bats revealed close homology to *T. cruzi marinkellei* (12) and *T. cruzi cruzi* (8), all of which are generalist parasites with many host species. *T. rangeli*, also a generalist, was present as a co-infection in 1 bat. The other type of haemoparasite was microfilariae of the genus *Litomosoides*. This genus is diversified in Neotropical bats, rodents and small marsupials (Brant and Gardner, 2000; Guerrero *et al.* 2002). *Artibeus jamaicensis* is a known host for 3 species of *Litomosoides* (Ubelaker *et al.* 1977).

Our study revealed that a bat infected with trypanosomes was more likely to harbour *Litomosoides* than a bat free of trypanosomes. This could

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be explained by the suppressive interaction of the immune responses versus trypanosomes and *Litomosoides*, and in the case of *Litomosoides* may be even through direct modulation of the host's immune system (Hoffmann *et al*. 2001; Hoerauf *et al*. 2005).

The body size and weight of *Artibeus jamaicensis* were not measurably influenced by the presence of *Litomosoides* or trypanosomes. This result supports prior observations in other bats where trypanosomes are regarded as non-pathogenic for their hosts, although there is some evidence of cysts formed by trypanosomes in the heart tissue of a variety of bats such as *Pipistrellus pipistrellus* and *Phyllostomus hastatus* (Molyneux, 1991). *Trypanosoma cruzi cruzi* isolated from bats, including *A. jamaicensis*, has shown low virulence in laboratory experiments when attempting to transfer the infection to mice (Hoare, 1972; Marinkelle, 1982). However, we cannot fully rule out that these two kinds of haemoparasites affect their bat hosts, because our sample is somewhat biased in that we could only survey active and hence probably healthy bats.

The elevated number of juvenile granulocytes (band cells) in bats with haemoparasites indicated intensified leukopoiesis (Lüllmann-Rauch, 2003). In bats infected with *Litomosoides*, irrespective of a co-infection with trypanosomes, eosinophilic granulocytes were significantly raised. As shown in humans and mice, filaric worms induce a T_H2 stimulation leading to massive eosinophile activation (Pichler *et al*. 1996; Le Goff *et al*. 1997). In *A. jamaicensis*, infection with trypanosomes correlated with an increased number of lymphocytes compared to healthy bats or bats with *Litomosoides*. Lymphocytes play a major role in the T_H1 pathway, the defence against trypanosomes. Co-infected bats showed changes in the blood count associated with both trypanosomes (T_H1) and microfilariae (T_H2) and therefore seem to be affected simultaneously. Even though there are other possible co-variates, we conclude that trypanosomes and *Litomosoides* triggered an obvious immune response in their host, compared to bats with no observed haemoparasites from the same environment.

Although we could not detect clear external signs of disease, we conclude from the blood counts that the two types of haemoparasite affect the physiology of their hosts. The activation of the immune system induced by parasite infection could have a negative impact on fitness as up-regulation of host immunity has been shown to reduce breeding success in birds (Ilmonen *et al*. 2000). In the case of *A. jamaicensis*, possible negative effects of infection with haemoparasites with regard to the performance of critical ecosystem services, i.e., seed dispersal, need to be studied more closely in the future.

Our results showed an effect of fragmentation on the multi-host parasite *Trypanosoma cruzi* (*sensu latu*) similar to the findings of Vaz *et al*. (2007). There was no such effect for *Litomosoides*, which is probably restricted to fewer host species (Guerrero *et al*. 2002).

Habitat fragmentation has lead to an overall reduction in the number of species and is contributing to high local densities of a few species. This is probably due to changes in the vegetation cover that favour adaptable species, such as *A. jamaicensis*. In bats captured on islands classified as small, isolated (Meyer *et al*. 2008; Guava, Guanabano), trypanosome prevalence was higher than in bats sampled on islands classified as larger or less remote (Leon, Cacao) and on the mainland sites. Our finding that trypanosome prevalence in *A. jamaicensis* was negatively correlated with the number of bat species supports the concept of the dilution effect (Ostfeld and Keesing, 2000*b*; Keesing *et al*. 2006; Vaz *et al*. 2007). A similar pattern has been shown in field studies combined with modelling approaches for the vector-transmitted Lyme disease (Ostfeld and Keesing, 2000*a*; LoGiudice *et al*. 2003). Bat-specific trypanosomes such as *T. cruzi marinkellei* also fit the conditions for the dilution effect because the known bat-specific trypanosomes seem to be generalists (Marinkelle, 1976). Our study species, *A. jamaicensis* is the most common of the more than 70 bat species recorded for the BCNM (Kalko *et al*. 1996; Meyer *et al*. 2008) and is a well-known reservoir for trypanosomes (Hoare, 1972; Marinkelle, 1982). In order to show that the dilution effect is really happening, the infection rate in the vectors on both mainland and islands needs to be examined closely in future studies.

Reduced species diversity appears to increase the transmission and prevalence of multi-host pathogens due to mechanisms that are not yet completely understood (Keesing *et al*. 2006). Human-induced alterations in environmental conditions causing impoverishment of faunal communities are known to enhance the propagation of zoonotic diseases (Ruedas *et al*. 2004).

Due to fragmentation, on both Guanabano and Guava the relative abundance of *A. jamaicensis* was very high (Meyer *et al*. 2008). High population densities induce stress (Martínez-Mota *et al*. 2007) and hormonal changes associated with stress can depress the immune system (Apanius, 1998). As illustrated in a theoretical model by Lafferty and Holt (2003), stress increases the impact of non-species specific diseases, such as trypanosomiasis. Additionally, dense populations pose a higher risk of contracting diseases with a higher possibility of transmission through increased contact with vectors and pathogens from host to host (Lyles and Dobson, 1993). A similar population-parasite pattern has been observed with gastrointestinal parasites in Colobus monkeys in forest fragments (Gillespie and Chapman, 2006).

Fragmentation also causes changes in habitat conditions, i.e. forest composition and cover (Leigh, 1999; Tabarelli *et al*. 2004) which are likely to affect trypanosome prevalence in *A. jamaicensis*. Compared to the mainland, the islands with the highest trypanosome prevalence (Guanabano, Guava), are covered by large numbers of palm trees, in particular *Scheelea zonensis* and *Oenocarpus mapora*, (Leigh, 1999). Those palm trees are preferred roosts of *A. jamaicensis* (Handley and Morrison, 1991; own observations) and harbour vectors of trypanosomiasis, particularly assassin bugs (Triatoma, Reduviidae) (Gaunt and Miles, 2000; Aufderheide *et al*. 2003). In particular, the palm *Scheelea zonensis* is strongly associated with incidences of Chagas' disease, as has been uncovered in Panama (Whitlaw and Chaniotis, 1978; Whitlaw, 1980). Furthermore, some reduviids (genus *Rhodnius*) feed mainly on vertebrates that visit palm trees (Gaunt and Miles, 2000). Thus, by roosting in palm trees, *A. jamaicensis* is extensively exposed to those vectors. Additionally, *A. jamaicensis* eats insects occasionally (Fleming *et al*. 1972). This might lead to the high trypanosome prevalence in areas with a high palm tree occurrence i.e. fragmented landscapes.

Bats from the other two, somewhat larger, islands Cacao and Leon, still had higher trypanosome prevalence than the bats pooled from the mainland. These islands seem to represent an intermediate state of fragmentation where the bat assemblages are more diverse than on the small, more isolated islands but lower than on the mainland (Meyer and Kalko, 2008*b*).

Litomosoides prevalence did not reveal a relationship with the capture locality of the bats. In contrast to trypanosomes, they are likely to be restricted to a few host species (Guerrero *et al*. 2002). Thus, fragmentation should not affect the distribution of microfilariae as strongly as multi-host parasites. Consequently, *Litomosoides* might be affected less by overall diversity loss than generalist parasites such as trypanosomes. It could be that some intrinsic factors on the part of the bat, such as age, are more important for the prevalence of *Litomosoides*, but we need to learn more about the natural history of this haemoparasite before firmer conclusions can be drawn.

Our study supports the proposed link between habitat fragmentation and increased prevalence of haemoparasitic infections, shown here for the common fruit bat, *A. jamaicensis*. More specifically, our results support the notion that species diversity, in addition to the habitat characteristics of individual fragments such as palm density, constitutes a driving factor behind the prevalence of trypanosomes as multi-host haemoparasites. This understanding is key for predicting and ideally, subsequently controlling the emergence and prevalence of disease (Walsh *et al*. 1993; Patz *et al*. 2000, 2004; Desjeux, 2001; Ruedas *et al*. 2004; Chapman *et al*. 2007). Especially in times of ever-growing human impact on ecosystems and global change it becomes even more important to have a grasp on fundamental factors affecting emerging diseases (Travi *et al*. 1994; Ostfeld and Keesing, 2000*b*; Chivian, 2001; Keesing *et al*. 2006). Conserving ecosystem functioning and ecosystem health is not only vital for ecosystems, but also for human health (Cook *et al*. 2004; Dobson *et al*. 2006).

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