Tropical rainforest flies carrying pathogens form stable associations with social non-human primates

Running title: Flies associate with monkey social groups

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Abstract

Living in groups provides benefits but incurs costs such as attracting disease vectors. For example, synanthropic flies associate with human settlements, and higher fly densities increase pathogen transmission. We investigated whether such associations also exist in highly mobile non-human primate groups (NHP). We studied flies in a group of wild sooty mangabeys (*Cercocebus atys atys*) and three communities of wild chimpanzees (*Pan troglodytes verus*) in Taï National Park, Côte d'Ivoire. We observed markedly higher fly densities within both mangabey and chimpanzee groups. Using a mark-recapture experiment, we showed that flies stayed with the sooty mangabey group for up to 12 days and for up to 1.3 km. We also tested mangabey associated flies for pathogens infecting mangabeys in this ecosystem, *Bacillus cereus* biovar *anthracis* (*Bc*bv*a*), causing sylvatic anthrax, and *Treponema pallidum pertenue,* causing yaws. Flies contained treponemal (6/103) and *Bc*bv*a* (7/103) DNA. We cultured *Bc*bv*a* from all PCR-positive flies, confirming bacterial viability and suggesting that this bacterium might be transmitted and disseminated by flies. Whole genome sequences of *Bc*bv*a* isolates revealed a diversity of *Bc*bv*a*, likely derived from several sources. We conclude that flies actively track mangabeys and carry infectious bacterial pathogens; these associations represent an understudied cost of sociality and potentially expose many social animals to a diversity of pathogens.

Keywords

Disease vector, sociality, polyspecific associations

INTRODUCTION

Living in social groups provides organisms a number of benefits but can also incur costs such as increasing the attraction of disease vectors. Possibly the strongest links between sociality and the risk of vector-borne disease in social living animals stems from comparative studies showing that malaria prevalence increases with group size across Neotropical primate species (Davies et al., 1991; Nunn et al., 2005). Synanthropic flies, which form close associations with human settlements and their livestock, have also been implicated in increasing disease risk in human settlements. A number of synanthropic fly species have been shown to serve as mechanical vectors for human pathogens (i.e., where a vector moves a parasite though is otherwise not necessary for the pathogen to complete its life cycle) and increasing fly densities have been shown to increase human disease risk (Banjo et al., 2005; Förster et al., 2007; Graczyk et al., 2001; Greenberg, 1971).

Synanthropic flies are involved in transmitting a broad array of pathogens. For example, they play a role in the transmission of many protozoan parasites (e.g., *Toxoplasma gondii*, Wallace 1971; *Giardia* spp., Markus 1980; *Cryptosporidium parvum*, Graczyk et al. 1999), bacteria (e.g., *Chlamydia trachoma*, Emerson et al. 2000; *Escherichia coli*, Iwasa et al. 1999; *Vibrio cholerae*, Echeverria et al. 1983), viruses (e.g., enteroviruses, Gregorio et al. 1972; Rift Valley fever virus, Turell et al. 2010), and helminth eggs (e.g., *Ascaris* spp. and *Trichuris trichiura*, Monzon et al. 1991). Whether the associations observed between synanthropic flies and humans are a product of the development of agriculture and a more sedentary lifestyle (Graczyk et al., 2001; Hassell et al., 2017), or whether such associations exist more broadly in hunter gatherer populations or wild non-sedentary non-human primate social groups and influence disease ecology is to our knowledge, currently unknown.

To address this knowledge gap, we examined the density of flies inside and outside a group of sooty mangabeys (*Cercocebus atys atys*) and two communities of chimpanzees (*Pan troglodytes verus*) living in

Taï National Park (TNP), Côte d'Ivoire and conducted a mark recapture experiment in the mangabey group to examine how a high density of flies is maintained as the group moves several kilometers each day through the tropical rainforest. Concurrently, we captured flies in the syntopic community of wild chimpanzees that came into proximity of the mangabey group to examine whether flies marked in the mangabey group might change association to a primate species. We tested flies found in association with the mangabey group for mammal DNA to see whether they had contact with other species and might move pathogens between species. We molecularly characterized the fly species present and examined whether specific fly species captured in association with these social groups might play a role in disease epidemiology by testing these flies for the DNA of pathogens known to infect wildlife in this ecosystem; specifically, for bacteria causing sylvatic anthrax (*Bacillus cereus* biovar *anthracis*: *Bc*bv*a*) (Hoffmann et al., 2017; Leendertz et al., 2004) and yaws (*Treponema pallidum pertenue*: *TPE*) (Gogarten et al., 2016; Knauf et al., 2018). To confirm that these flies containing *Bc*bv*a* DNA could be involved in pathogen transmission and contained viable bacteria, we attempted to culture *Bc*bv*a* from these flies and then sequenced the full genome of these isolates. To examine a potential epidemiological link between the *Bc*bv*a* in flies and that killing mangabeys, we also cultured *Bc*bv*a* from a mangabey that died during the study period in the same area and sequenced its full genome.

Sylvatic anthrax, caused by *Bc*bv*a*, is a persistent and widespread cause of death in a broad range of mammalian hosts in this ecosystem and was responsible for more than 38% of wildlife mortality observed over 26 years (Hoffmann et al., 2017; Leendertz et al., 2004). *Bc*bv*a* was the likely cause of death for 11 of 23 mangabeys and 31 of 55 chimpanzees for which necropsies were performed in Taï National Park and seroprevalence rates are extremely low in these species, suggesting *Bc*bv*a* is highly lethal (Hoffmann et al., 2017; Zimmermann et al., 2017). Furthermore, *Bc*bv*a* DNA was detected in more than 5% of flies sampled throughout TNP and many of these flies were shown to contain viable spores (Hoffmann et al., 2017). In non-sylvatic ecosystems, it has been suggested that flies mechanically spread spores from *Bacillus*

anthracis, the closely-related causative agent of classical anthrax, from carcasses through the environment, potentially leading to subsequent transmission (Blackburn et al., 2014; Fasanella et al., 2010). Little is known about the transmission and persistence of sylvatic anthrax.

Non-human primates in TNP are also infected with *TPE* and present with severe lesions (Figure 1A; Gogarten et al., 2016; Knauf et al., 2018). Many mangabeys in the study group (referred to as the Audrenisrou group) presented with yaws symptoms during the study period (16% of individuals; data not shown) and full *TPE* genomes generated from lesion samples confirmed this pathogen is present in animals collected in the study group with these symptoms (Knauf et al., 2018). Chimpanzees in this ecosystem also appear to be infected with *TPE*, with next generation sequencing data from chimpanzee bones confirming a *Treponema pallidum* pathogen is present in these communities, though to date no samples from lesions are available to confirm infections (Gogarten et al., 2016). Flies have long been hypothesized to play a role in the epidemiology of yaws, with studies showing that flies can carry treponemes from lesions (Kumm, 1935; Satchell et al., 1953) and in experimental conditions, that flies transmitted the parasite from one host to another host when feeding on lesions (Kumm et al., 1936). Further, a high proportion of flies captured in two national parks in Tanzania, where wild olive baboons (*Papio anubis*) are infected with *TPE*, were found to contain *Treponema pallidum* DNA (Knauf et al., 2016); based on the low variability of the genomic regions of *Treponema pallidum* examined in this study, it was not possible to definitively determine which subspecies of *Treponema pallidum* was present in these flies, though the authors argue their results suggests that flies often come into contact with the spirochete on these baboons as there is no evidence for other *Treponema pallidum* subspecies circulating in this ecosystem (Knauf et al., 2016).

MATERIALS AND METHODS

(a) Study site

This study was conducted on flies that associate with wild primates in TNP, Côte d'Ivoire (6°20'N to 5°10'N and 4°20'W to 6°50° W). TNP represents the largest remaining primary forest in West Africa and the wild non-human primate populations present in this ecosystem represent some of the best-studied populations in the world; studies on the chimpanzees and monkeys of TNP were initiated in 1979 and 1989 respectively (Boesch et al., 2000; McGraw et al., 2007) and a veterinary program that started in 2001 has targeted a broad array of pathogens associated with these populations (Gogarten et al., 2014; Hoffmann et al., 2017; Leendertz et al., 2006; Rich et al., 2009). We focused on a group of sooty mangabeys habituated to human observers in November 2012 (the Audrenisrou group), which consisted of ~60 individuals during the study period (Gogarten et al., 2018; Mielke et al., 2017). We also captured flies near a habituated chimpanzee community (the North group) with 17 individuals (in August 2013), whose territory overlaps that of the mangabey group, and two neighboring habituated chimpanzee communities (the South and East groups, containing 41 and 36 individuals respectively in December 2018).

(b) Fly trapping to assess density inside and outside of the primate groups

A large diversity of fly species is found in Taï National Park (Hoffmann et al., 2017). Genera present contain species that are known to be necrophagous, coprophagous, hematophagous, or myiatic, and their diets can be flexible and opportunistic, including different food types depending on what is available, though the life cycles and ecology of these sylvatic fly communities are poorly described. Flies were caught using either custom-made traps (described in: Hoffmann et al., 2017) placed over a commercial attractant based on animal proteins that mimic a decaying carcass (hereafter: *synthetic carcass baited traps*; Unkonventionelle Produkte Feldner, Waldsee, Germany) or using feces as an attractant. Flies were not attracted to fecal samples once cooled; to circumvent this limitation and ensure sampling conditions were similar at all distances from the primate group, a thermos full of hot water was used to warm fecal samples in a plastic bag placed at the top of the thermos (Figure S1). Flies were trapped using a clear plastic bag lowered over

feces and startled flies would then fly and walk upwards in the bag (hereafter: *feces baited traps*). Both types of traps were left open for 20 minutes and flies were euthanized with ether. Flies from a given trap were stored at ambient temperature on silica in 50 ml Falcon tube containing up to 20 flies until they were transported back to the Robert Koch Institute and subsequently stored at 4°C. Flies caught with synthetic carcass baited traps did not have contact with the bait, while flies captured with feces baited traps were in contact with feces prior to capture. A researcher remained next to the traps throughout the entire experiment to discourage curious NHP from coming into contact with the traps. Our aim was to avoid any potential increase in disease risk for the primates through exposure to flies in the traps or through exposure to human microorganisms on the traps themselves. Had NHP come into contact with the traps, we would have immediately stopped the experiment. Throughout these experiments no mangabeys or chimpanzee came into contact with the fly traps.

During five days between July 30th and August 3rd 2013, six fresh fecal samples were collected in the morning at the mangabey sleeping tree. Several hours after the mangabeys left the sleeping site, trapping of flies using feces bait traps was conducted at 1km from the group, 500m from the group, and within the mangabey group. These trapping distances were selected because individuals are usually within a range of 100m of one another - we hoped that 500m and 1000m were sufficiently far from the study group that we would be clearly outside of it, while enabling us to travel the distance quickly so we could find the group again after sampling. On each day, two independent traps were set at different locations at each of the three distances, for a total of six trapping events per day. To avoid biasing sampling, the order of sampling each day was randomized. We repeated this experiment using synthetic carcass baited traps on 10 additional days between May 13th and May 29th, 2014.

We repeated these fly density experiments with two communities of chimpanzees (South and East Groups) using feces baited traps in December 2018. Based on our results with the mangabeys and because of the

larger home range sizes of chimpanzees that makes finding the group after you leave it difficult, we were not able to also set traps at 1000m from the group and traps were almost always first set in the chimpanzee group and then 500m from the group.

(c) Mark and recapture of flies

Flies were collected in the morning underneath the mangabey sleeping tree using a clear plastic bag placed over fresh feces (Figure 1B). Flies were placed in a cooler with ice packs to anesthetize them. Inspired by studies marking lice on lemurs (Zohdy et al., 2012), we marked flies on the posterior abdomen or thorax with a small amount of nail polish from a pipet tip, with a distinct color used at each marking location. GPS coordinates were taken at the point of marking. Flies were marked with five different colors on five days (N_{marked}=1,591 flies; July 19th, 21st, 22nd, 23rd, and 27th). Flies captured after the initial marking day were checked for nail polish and the GPS point, number of marked, and the number of unmarked flies were recorded during each capture event ($N_{recapture\ effort}$=3,164 flies). On August 1st, a party of 11 chimpanzees from the North group moved through the mangabey group's territory at the same time we were conducting the mark recapture experiment; the North group has a much larger but overlapping territory with the sooty mangabey Audrenisrou group (Boesch et al., 2000). On the day after they passed close to the mangabey group, we sought to examine whether flies marked in the mangabey group might have found their way into the chimpanzee party by opportunistically collecting flies (*N*=166) with feces baited traps.

(d) Flies in the social group after sleeping near a decaying carcass

On May 2nd, 2014 the mangabey group slept in a tree near a dead duiker carcass (Figure 1C); to examine whether flies on the carcass might pick up pathogens from carcasses and then travel with the group, on May 3rd, flies were captured over feces at 9:00 a.m. as the mangabeys were leaving the tree, and again at 10:00 a.m., 12:00 a.m., 1:17 p.m., and lastly at 5:30 p.m. when the mangabeys entered their sleeping tree. A chimpanzee died elsewhere in the forest on the same day that the duiker carcass was found with the

mangabeys and the veterinarian on site prioritized performing the necropsy on the chimpanzee and so no necropsy was performed on this dead duiker carcass. We did however perform necropsies from duikers in the study area on April 11th, 17th, 28th and May 13th 2014, for which *Bc*bv*a* was confirmed as the cause of death (Hoffmann et al., 2017), demonstrating that anthrax was causing mortality in this duiker population. A necropsy on a mangabey in the study group that died on May 10th 2014 confirmed that *Bc*bv*a* was the likely cause of death, showing that mangabeys were being exposed to anthrax through some route (Hoffmann et al., 2017). This sample set allowed for an examination of a potential epidemiological link between *Bc*bv*a* in flies present in the group before the mangabey's death (N=3) and the *Bc*bv*a* that killed the mangabey, but also with flies that were captured in the group after this mangabey's death (N=4).

(e) Molecular analyses

Extraction

DNA was extracted from a subset of individual flies captured using the synthetic carcass baited traps (N=45) and feces baited traps (N=33) in the mangabey group. DNA was also extracted from a subset of flies captured over fresh mangabey feces on the morning after the group slept near a duiker carcass (N=5 flies/capture events, total 25 flies). DNA was extracted from flies using the GeneMATRIX Stool DNA Purification Kit (Roboklon, Berlin, Germany). Prior to homogenization with a Fast Prep® (MP Biomedicals, Santa Ana, CA, USA) each fly was cut into smaller pieces with sterilized scissors, but otherwise extraction followed the manufacturer's instructions. DNA was extracted from the spleen of the dead mangabey using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). DNA concentration was measured using a Nanodrop (Thermo Scientific, Waltham, MA, USA) and extracts were stored at -20°C.

Fly species identification

To determine the fly species for this subset of flies we Sanger sequenced the COI fragment of these 103

flies captured in the sooty mangabey group following (following: Folmer et al., 1994; details in Supplementary material). We aligned the resulting 96 sequences using Muscle (Edgar, 2004) as implemented in SeaView v4 (Gouy et al., 2009). This alignment was used for phylogenetic analyses in both maximum likelihood (ML) and Bayesian frameworks. We generated a ML tree using PhyML v3 with smart model selection (PhyML-SMS; Guindon et al., 2010; Lefort et al., 2017). We used a full optimization approach, and the tree search used subtree pruning and regrafting and the Bayesian information criterion for model selection. Branch robustness was assessed with Shimodaira-Hasegawa-like approximate likelihood ratio tests (SH-like aLRT: Anisimova et al., 2011). We also ran Bayesian Monte Carlo Markov chain (BMCMC) analyses with BEAST v1.8.2 (Drummond et al., 2012) using the nucleotide substitution model identified by PhyML-SMS, a lognormal relaxed clock (uncorrelated) and a birth-death speciation model. Multiple BMCMC runs were performed; we checked that runs converged and that the posterior was properly sampled using Tracer v1.6 (http://tree.bio.ed.ac.uk/software/tracer/). We combined posterior sets of trees using LogCombiner v1.8.2 (distributed with BEAST) and identified the maximum clade credibility tree using TreeAnnotator v1.8.2 (distributed with BEAST). Branch robustness was assessed with posterior probabilities. We also performed a ML analysis on a reduced dataset only comprising unique sequences (N=44), which we identified using FaBox v1.41 (Villesen, 2007). The resulting tree was used for a species delimitation analysis using a Bayesian Poisson tree processes model (bPTP; Zhang et al., 2013). We performed taxonomic assignment of the respective molecular operational taxonomic unit (MOTU) using BLAST (Altschul et al., 1990) and the non-redundant nucleotide database of NCBI.

Mammal testing

To determine whether flies associated with this social group were exposed to mammal DNA, we used a pan-mammal PCR targeting a 130bp region of the mitochondrial 16S DNA (with primers and blockers described in; Boessenkool et al., 2012; Taylor, 1996; full protocol described in; Calvignac‐Spencer et al. 2013b).

Chromatograms were evaluated using Geneious Pro v 8.1.3 and cleaned sequences were assigned to mammalian species using BLAST.

Pathogen screening

Bc*bv*a

Following Hoffmann et al. (2017) all DNA extracts were first tested for *Bc*bv*a* in duplicate with a real-time PCR targeting *pag* (gene coding for the protective antigen; PA) located on the pXO1 plasmid. Positive samples were then tested in duplicate using real-time PCRs targeting *capB* (gene coding for capsule synthesis) located on PXO2 and Island IV, a chromosomal marker unique to *Bc*bv*a*, which can be used to discriminate from *Bacillus anthracis*. Standards of a known concentration were available for all three assays allowing us to estimate template copy numbers and all assays were consistently able to detect 10 template copies. All real-time PCR runs were conducted using a Stratagene qPCR MX3000 cycler (Stratagene, Lajolla, CA, USA) and fluorescence signals were quantified with the software MXPRO (www.genomics.agilent.com). Conservatively, only samples positive in duplicate in all three *Bc*bv*a* assays were considered positive for *Bc*bv*a* DNA for the analyses presented below.

From all *Bc*bv*a* positive flies culture was attempted under biosafety level 3 conditions. Half of the fly mush that remained after DNA extraction was plated onto the following plates: Columbia blood agar (Oxoid, Wesel, Germany), blood-trimethoprim agar (1.6 mg trimethoprim, 6.4 mg sulfamethoxazole, 20 mg polymyxin B per liter agar medium) and Cereus Ident agar (Heipha Diagnostica, Eppelheim, Germany) with the chromogenic substrate 5-bromo-4-chloro-3-indoxyl-myoinositol-1-phosphate (Klee et al., 2006). In addition, a 10 µl aliquot of the mush was diluted 1:10 in sterile NaCl, heat treated for 30 min at 65°C and then plated on the plates described above. Cultures were incubated at 37°C and monitored daily and any morphologically suspicious colonies were sub-cultured, heat-inactivated and tested in real-time PCR as described above. We followed the same procedure for culturing *Bc*bv*a* from the mangabey that died on May

273 10th, 2014. *Bc*bv*a* was retrieved from all native and heat treated samples, indicating the presence of heat-
274 resistant spores.
275
276 Libraries for whole-genome sequencing were prepared using the Nextera XT Sample Preparation Kit
277 (Illumina) and Nextera XT Index Kit (Illumina) using 1 ng of heat extracted DNA from the isolates as input.
278 We used AMpure XP beads (Beckman Coulter) for PCR clean-up. Final concentrations were assessed using
279 a Qubit High Sense Double Stranded DNA Assay kit (Invitrogen, Carlsbad, CA, USA) and equimolar
280 amounts of libraries were pooled and sequenced in two runs using the Illumina MiSeq platform with v3
281 chemistry (2x300 bp) and the Illumina NextSeq 500 platform (2x150 bp). Reads from both runs were
282 combined, quality filtered using Trimmomatic, setting the quality score to 30 over a sliding window of four
283 bases, with a minimum read length of 40 bp for the surviving reads. Filtered reads were then mapped to a
284 *Bc*bv*a* chromosomal reference genome (NC _014335) using BWA-MEM (Li, 2013), and deduplicated using
285 Picard's MarkDuplicates. Alignments with a MAPQ smaller than 30 and a mapping length lower than 30
286 were removed using SAMtools (Li et al., 2009) and the BBMap reformat script
287 (https://github.com/BioInfoTools/BBMap/blob/master/sh/reformat.sh). Variants were called using the
288 Variant Calling Pipeline (https://gitlab.com/RKIBioinformaticsPipelines/VariantCalling/) that uses GATK
289 (McKenna et al., 2010) and SnpEff (Cingolani et al., 2012) A summary of the reads generated and the
290 coverage for each isolate are available in Table S1. We considered a variant as present if it was present in
291 95% of reads and at a minimum 10X coverage. To compare the dead mangabey *Bc*bv*a* isolate with the
292 *Bc*bv*a* isolates from flies and accommodate uneven depth across isolates, we took all variants that were
293 confidently assigned (present in 95% of reads and at a minimum 10X coverage) in the mangabey and
294 assessed whether they were also present at those positions in the fly isolates (present in 95% of reads and at
295 a minimum 10X coverage). We also looked at all other variants that were confidently assigned (present in
296 95% of reads and at a minimum 10X coverage) in the fly isolates and determined whether they were present
297 in the mangabey isolate (present in 95% of reads and at a minimum 10X coverage). For each fly, we then

had an assessment of the variants shared with the dead mangabey, the variants confidently not shared with the dead mangabey, and unknown positions where sequencing depth was not sufficient to confidently call a variant in either the fly or dead mangabey isolate. We previously described *Bc*bv*a* diversity within carcasses and found that isolates differed by a maximum of 2 chromosomal SNPs (Hoffmann et al., 2017), suggesting flies differing from the dead mangabey isolate by ≤2 variant positions are either epidemiologically linked or stemmed from the same source.

Treponemal DNA

DNA extracts were tested using a PCR amplifying a 67 bp DNA fragment, including primers from the DNA polA gene (described in: Leslie et al., 2007). An additional PCR using fusion primers was performed to append M13F/R sequences to first-round amplicons and thereby enable their sequencing (as described in; Gogarten et al., 2016). We tested the sensitivity of this assay and found it was able to detect as few as 10 template molecules spiked into fly extracts (see Supplementary material for details). To add further support that *Treponema pallidum* was present, we performed a semi-nested PCR amplifying a 189 bp sequence of the *Treponema pallidum* cfpA gene (Harper et al., 2012; See Supplemantary material for details; Harper et al., 2008). Products were sequenced using Sanger sequencing and sequences were compared to publicly available sequences in NCBI through BLAST (Altschul et al., 1990). We assessed the sensitivity of this assay and found it was able to detect 1000 template molecules spiked into fly extract, but not able to consistently detect 100 template molecules spiked into fly extracts (see Supplementary material for details). Even tissue samples positive for *Treponema pallidum* have been positive in the short polA assay and then negative in the cfpA assay, despite having observed the spirochete in electron micrograph and ultimately being able to capture the whole genome using hybridization capture methods, further highlighting the low sensitivity of the semi-nested cfpA assay (unpublished data: SCS, JFG, FHL; Knauf et al., 2018).

The regions amplified by both the polA and semi-nested cfpA assays are identical in closely related

Treponema paraluisleporidarum, the closest relative of *Treponema pallidum* described to date (Šmajs et al., 2011), as well as other *Treponema pallidum* subspecies. To date, *Treponema paraluisleporidarum* has only been found in rabbits and hares, and no rabbits or hares have been detected in flies in TNP despite extensive sampling of flies (unpublished data: CH, CSC, FHL; Hoffmann et al., 2017), suggesting rabbits and hares are unlikely sources of treponemal bacteria in these flies. Similarly, no other *Treponema pallidum* subspecies have been described in the wildlife in this ecosystem. Because of the variation in sensitivity of the two assays used here and the fact that no hosts for *Treponema paraluisleporidarum* have been detected in flies in TNP, samples that yielded a *Treponema* sequence in the polA assay were tentatively considered *TPE* positive for the analyses presented below. *TPE* is microaerophilic and cannot grow on standard culture media (Edmondson et al., 2018), requiring direct culture of the treponemes in laboratory animals. We were unable to perform such culture experiments, so it was not possible to determine the viability of the *TPE* and these results should be interpreted with caution.

(f) Statistical analyses

To test whether the number of flies captured at synthetic baited or feces baited traps was influenced by the distance from the mangabey group, we used a generalized linear mixed effect models using a Poisson error structure and log link function (Baayen, 2008), with the number of flies caught at each trap as the response variable. Models were fitted separately for synthetic carcass baited traps and feces baited traps. Into these models we included the distance from the social group as a categorically coded fixed effect and trapping day as a random effect. To control for the effect of the ID of the person setting the trap and the hour the trap was set, these were included as further fixed effects. The hour the trap was set was approximately symmetrically distributed and z-transformed. To model the overdispersion that was evident in these models, we included trap ID as an observation level random effect (Harrison, 2014). Models were fitted in R using the function glmer in the R package *lme4*, using 1,000 parametric bootstraps. Samples used for the feces baited trap model consisted of 30 traps set over 5 days, while for the synthetic carcass baited traps this

consisted of 60 traps set over 11 days.

As an overall test of the effect of the distance from the mangabey group on the number of flies caught by a trap, we compared the full model with a null model lacking this fixed effect but comprising the same random effects structure and control fixed effects (Forstmeier et al., 2011) using a likelihood ratio test (Dobson, 2002). We checked whether the assumptions of normally distributed and homogeneous residuals were fulfilled by visually inspecting a qqplot and residuals plotted against fitted values; both indicated no obvious deviations from these assumptions for either feces baited traps or synthetic carcass baited traps.

To rule out problems of collinearity we determined Variance Inflation Factors (VIF: Field, 2005) for the standard linear model excluding random effects, which for feces baited traps revealed VIF of 1.02 for distance from the mangabey group, 1.00 for ID of capturer, and 1.02 for the hour the trap was set. For the synthetic carcass baited traps this revealed a VIF of 1.01 for distance from the mangabey group, 1.02 for ID of capturer, and 1.03 for the hour the trap was set, suggesting there were no issues with collinearity for either model.

To examine whether the number of flies captured with feces baited traps was higher in the chimpanzee groups than 500m away from the group, we ran a separate paired t-tests for each of the two groups. We log transformed the number of flies captured to improve normality of this variable. Small sample sizes (10 and 9 traps in the East and South groups respectively, along with 10 and 9 paired traps 500m away from the East and South groups respectively) precluded the use of generalized linear mixed effect models including time of trap set and date as random effects or an analysis of the impact of variation in party size on fly density.

To test for an association between character states (i.e., the type of attractant used to capture the fly, whether the fly contained mammal DNA, *Bc*bv*a* positivity, or *Treponema pallidum* positivity) and the fly phylogeny, we used Bayesian Tip-association Significance testing (BaTS). This approach tests whether any given taxon on the tree is more likely to share a character state with a sister taxon than expected by chance. BaTS uses the posterior sets of trees generated through the BMCMC analysis described above and incorporates the phylogenetic uncertainty arising from the data into its test of phylogeny-trait associations (Parker et al., 2008).

RESULTS

(a) Fly density

Overall there was a clear impact of the distance from the mangabey group on the number of flies captured for both synthetic carcass baited traps (likelihood ratio test comparing full and null model: χ^2=19.929, df=2, P<0.001; Table S2) and feces baited traps (χ^2=41.443, df=2, P<0.001; Table S3). More specifically, the number of flies captured at each trap type was higher within the group than outside the group (Figure 2A and B; Table 1). Similarly, in both chimpanzee groups, there were more flies in the group than 500m away from the group (East group: $\bar{x}_{0m}$=19.1 flies, $\bar{x}_{500m}$=3.8 flies, paired t-test, P<0.001, *t*=12.974, df=9; South group: $\bar{x}_{0m}$=17.7 flies, $\bar{x}_{500m}$=2.4 flies, paired t-test, P<0.001, *t*=6.556, df=8; Figure 3 and Table S4).

(b) Mark recapture

Of the 1,591 flies marked in the mangabey group, 51 (3.2% of flies that were marked) were recaptured using a recapture effort of 3,164 in the mangabey group (Table S5). These flies were captured up to 1.3 km from the point of marking (mean distance traveled = 703m, SD = 297m) and were recaptured with the group up

to 12 days after marking (mean days since marking = 3.4 days, SD = 2.6 days; Figure 4). The fly density experiment using feces as a bait described above was carried out shortly after the marking for the mark recapture experiment had finished. Specifically, the fly density experiment using a feces bait experiment was carried out 3 to 7 days after the last fly was marked in the mangabey group. During this fly density experiment using feces as a bait, 0% of flies (0/92) captured at 500m and 1000m from the group were marked. In contrast, 2.05% (11/534) of flies captured in the group on the same days were marked. On August 1st, 2013, one marked fly was recaptured with the chimpanzee group that passed through the mangabey group's territory and close to the mangabey group, 628m from where it was originally marked and nine days after having been marked in the mangabey group.

(c) Fly species

Our analysis suggested that the 96 flies for which good quality COI sequences were generated (poor quality sequences were generated for 7 flies) belonged to 14 putative species. Most flies were assigned to the family Muscidae (45.8%), Calliphoridae (35.4%) and Sarcophagidae (8.3%), while the remaining 10.4% of flies could not be assigned to a family based on BLAST results (Table 3). The BaTS analysis suggested that the type of attractant used to capture a fly was structured on the phylogeny (Table 4). While representatives of nearly all molecular operational taxonomic units (MOTUs) were captured using both feces and synthetic carcass baited traps, MOTU ratios measurably differed depending on the bait. For example, 60% of the flies attracted to feces belonged to MOTU 1 (family Muscidae); only 30% of the flies attracted to synthetic carcass bait belonged to the same MOTU.

(d) Mammal DNA

Sooty mangabey DNA was found in 40 of the 53 (tested) flies captured over feces, while only 7 of 45 (tested) flies captured with a synthetic carcass baited trap contained mangabey DNA. As most of the flies that were captured over feces belonged to the family Muscidae, mammal positivity was also highest among

this group and the BaTS analysis suggested that mammal positivity was structured on the fly phylogeny (Table 4). Duiker DNA was detected in three flies captured over mangabey feces the day after the mangabey group slept near a duiker carcass.

(e) Pathogen screening

*Bc*bv*a*

12 of the 98 flies tested in the mangabey group were positive in duplicate reactions for the *Bc*bv*a* PA qPCR, with copy number estimates per µl ranging from 5.3 to 1228.0 (Table 1 and S5). Of these positive flies, 8 tested positive in duplicate reactions for the CapB qPCR, with copy number estimates per µl ranging from 6.5 to 1175.5. Seven of these positive flies were also positive in the *Bc*bv*a* Island IV qPCR with copy numbers ranging from 3.38 to 1720.0. The highest *Bc*bv*a* copy numbers were observed in flies captured in the mangabey group with feces on the day after they slept over a dead duiker carcass (Table S6). None of the flies positive for *Bc*bv*a* DNA in all three assays contained mangabey DNA (Figure 5, Table S6) but several contained duiker DNA. Flies containing *Bc*bv*a* DNA were captured with both bait types and all belonged to the family Calliphoridae (Figure 5, Table S6), though the BaTS analysis did not detect a pattern of *Bc*bv*a* positivity on the fly phylogeny. This may be due to the small number of flies positive for *Bc*bv*a* in all three assays. It was possible to culture *Bc*bv*a* from the seven flies positive in the three assays and from the dead mangabey.

In the dead mangabey *Bc*bv*a* isolate 1903, we identified 62 variant sites (Table S7; details on sampling timeline: Figure 1D). The *Bc*bv*a* isolate from fly 3465 only differed from the dead mangabey at a single one of these variant sites; all additional 7 variants identified in the *Bc*bv*a* isolate from fly 3465 could not be confidently called in dead mangabey isolate but were present at lower coverage in this isolate (Table S8). Thus, these isolates were nearly identical, differing by only a single SNP. In contrast, all other flies were more different from the mangabey isolate; the *Bc*bv*a* isolates from fly 3488, 3498, 3487, 3495, 3496, and

3464 respectively differed at 8, 27, 31, 33, 34 and 37 of the mangabey isolate's 62 variant sites. The mangabey isolate differed at 5 of the additional variants identified in the *Bc*bv*a* isolate from fly 3488, while from flies 3498, 3487, 3495, 3496, and 3464 respectively the mangabey isolate 1903 differed at 9, 5, 13, 13, and 5 of the additional variant sites, further highlighting the variability of these isolates (Table S8).

Treponema pallidum

Six of 98 (6.12%) flies tested in the mangabey group were positive with the PCR for a short fragment of the polA gene (Figure 5, Table S6), with sequences showing 100% identity to published *Treponema pallidum* strains. Only one of those six flies tested positive for a longer fragment of the cfpA gene. The low sensitivity of the cfpA assay may be responsible for the low positivity of this confirmatory test. Of these 6 flies containing *Treponema pallidum* DNA, 4 contained sooty mangabey DNA, including the fly positive in both *Treponema pallidum* assays (Figure 5, Table S6). *Treponema pallidum* positive flies were captured using both feces and synthetic carcass baited traps (Figure 5, Table S6) and a number of different fly MOTUs contained *Treponema pallidum* DNA. The BaTS analysis suggested there was no pattern of *Treponema pallidum* positivity on the fly phylogeny (Figure 5, Table 4), though this again may be due to the low number of flies containing this pathogen's DNA.

DISCUSSION

Here we document a diverse fly community associated with a mangabey social group containing putative species belonging to fly genera that are reported to be opportunistically necrophagous, coprophagous, hematophagous, and/or myiatic. We found that these flies were at a higher density within the mangabey group than at different distances away from the group. We observed a similar pattern in two neighbouring chimpanzee communities, with significantly higher fly densities inside than outside groups. Further, flies

471 marked in the mangabey group moved with the group for up to 12 days and for a straight-line distance of
472 1.3km through the rain forest; mangabey groups do not move linearly through this environment, suggesting
473 the actual distance moved by these flies is larger. If the process of marking flies impacted their survival and
474 mobility, our estimates likely represent a further underestimation of the maximum duration and distance
475 travelled by flies in these associations. The finding of mangabey DNA in most of the flies captured in the
476 mangabey group is not surprising given that feces baited flies were observed coming into contact with feces
477 prior to being captured, though many flies captured with the synthetic carcass baits also contained mangabey
478 DNA. This suggests that some of the flies captured with the synthetic bait had come into contact to the
479 mangabeys or their excrement. Flies captured using mangabey feces were less diverse than those captured
480 with synthetic carcass baits, which supports the notion that some fly species prefer certain foods, though all
481 fly MOTUs that were captured more than three times, were capture with both feces and synthetic carcass
482 baits.
483
484 Our results suggest that similar to synanthropic flies that increase human disease risk (Banjo et al., 2005;
485 Förster et al., 2007; Graczyk et al., 2001; Greenberg, 1971), flies associating with NHP social groups have
486 the potential to increase NHP disease risk. Flies captured in the mangabey group contained the DNA of two
487 pathogens that infect wildlife in this ecosystem, *Bc*bv*a* causing anthrax and likely *TPE* causing yaws,
488 suggesting they may play a role in the ecology and persistence of these pathogens. Caution is warranted in
489 the interpretation of the *Treponema pallidum* result, as the regions amplified by the assays for *TPE* used
490 here are identical in the closely related *Treponema paraluisleporidarum*, as well as other pathogenic
491 *Treponema pallidum* subspecies. To date, *Treponema paraluisleporidarum* has exclusively been described
492 from rabbits and hares and no rabbits or hares have been detected in flies in this ecosystem, despite an
493 extensive sampling of flies (unpublished data: CH, CSC, FHL; Hoffmann et al., 2017). Thus, rabbits and
494 hares are unlikely to represent a source of treponemal bacteria found in these flies. The other *Treponema*
495 *pallidum* subspecies described to date have not been found in wildlife species, though it is possible that

other wildlife are infected with these or other treponemes that have not yet been described. The mangabey group in which the flies were captured was suffering from a continuous TPE outbreak, with ~16% of animals showing visible symptoms, which could represent a source of the treponemal DNA in the flies; further research is needed to definitively link the treponemes detected in flies with infections observed in these mangabeys. To this end, the development of sensitive PCRs allowing for a subspecies distinction of these treponemes will be extremely helpful, as would systems for culturing TPE (Edmondson et al., 2018). Laboratory infection studies with model organisms using flies carrying treponemes in the concentrations observed in the wild would ultimately be necessary for determining whether these flies can really lead to infection in another individual when flies come into contact with other animals, for example while feeding on open wounds (Kumm et al., 1936).

The fact that we were able to culture *Bc*bv*a* from these flies captured in the mangabey group, confirms they contain viable spores and could potentially be spreading *Bc*bv*a* to the mangabeys and their surroundings. In fact, the highest concentration of *Bc*bv*a* DNA (>1000 copies/ul of DNA), some of the highest copy numbers we have observed in flies in this forest (Hoffmann et al., 2017), were found in flies captured during the day after the mangabey group slept near a decaying duiker carcass, suggesting flies may move pathogens from carcasses into monkey groups. Indeed, it was possible to culture *Bc*bv*a* from several of these flies captured on the day the mangabeys slept near a duiker carcass, and these flies both contained duiker DNA and their *Bc*bv*a* isolates were nearly identical, suggesting they were exposed to *Bc*bv*a* at a single carcass. A necropsy on a mangabey in the study group that died several days after sleeping near the decaying carcass confirmed that *Bc*bv*a* was the likely cause of death, though comparisons of the *Bc*bv*a* isolate from the dead mangabey with those obtained from flies captured in the week prior to the mangabey's death, suggested these cases were not epidemiologically linked. The *Bc*bv*a* isolate from one fly captured a week after the mangabey's death had only a single variant compared to the dead mangabey isolate. It is not possible to know whether this fly picked up this *Bc*bv*a* variant from the mangabey carcass itself, or whether the fly and mangabey

both picked up that *Bc*bv*a* variant from some other source.

Flies in non-sylvatic ecosystems have been shown to mechanically spread spores from carcasses through the environment and contaminate plant surfaces, potentially increasing exposure of other animals (Blackburn et al., 2014; Fasanella et al., 2010). This is of particular concern if the flies follow a social group through the forest to feeding sites where they could contaminate food items (e.g. with their regurgitation or feces). While *Bc*bv*a* was the likely cause of death for 11 of 23 mangabeys for which necropsies have been performed in TNP (Hoffmann et al., 2017), there was not a major mangabey die off while this study was carried out. With so many flies in the mangabey group carrying high concentrations of viable *Bc*bv*a*, it is perhaps surprising that more mangabeys were not dying of anthrax. Laboratory studies using small animal models (e.g., Brézillon et al., 2015) are needed to confirm whether the amount of viable *Bc*bv*a* spores or vegetative bacilli carried by flies are sufficient to cause mortality in a host directly (e.g., through consumption of flies by monkeys or apes or consumption of foods covered in fly vomit spots). Flies following monkeys might explain how arboreal monkey species are exposed to *Bc*bv*a* spores in this ecosystem (Hoffmann et al., 2017). From the perspective of a carrion fly, moving pathogens from carcasses to living animals might increase a fly's fitness by increasing the number of carcasses in the environment for itself or its offspring. This raises the interesting prospect that there could be selection for flies to be able to transmit pathogens efficiently from carcasses to animal groups.

Further research is needed to assess whether these fly associations are found more broadly in different NHP species and in other ecosystems. It will be interesting to understand how these flies are able to track NHP and maintain their associations. Many hematophagous vectors (e.g., mosquitos) are attracted to their hosts via their carbon dioxide production (Gillies, 2009; Kellogg, 1970) and it will be interesting to test whether NHP-associated flies are using similar mechanisms. The finding of two chemosensory receptors that mediate carbon dioxide detection in *Drosophila* suggests the ability to detect and track carbon dioxide

production could be present in other insects as well (Jones et al., 2007). However these flies are able to maintain such an association, the persistent fly community around a NHP social group could limit fly dispersion and movements between groups and species limiting pathogen transmission. Our data do not, however, support such a hypothesis; duiker DNA and *Bc*bv*a* DNA was found in flies in this group, a fly marked in the mangabey group shifted to the chimpanzee group, and *Bc*bv*a* DNA in flies captured outside a social group (Hoffmann et al., 2017) had similar *Bc*bv*a* prevalence to that found inside the group in this study, all suggesting movement of flies into and out of association with particular primate groups.

Synanthropic flies have also been suggested to serve as a vector between livestock and humans (Rosef et al., 1983). Determining whether NHP-associated flies can move out of the forest and into surrounding human populations is an important area of future research. We conducted a small-scale preliminary analysis of the mammalian and *Bc*bv*a* DNA found in 45 flies captured in a village near Taï National Park. Though we did not detect any *Bc*bv*a*, we did detect four wildlife species (*Cephalophus* sp., *Praomys* sp., *Piliocolobus badius*, and *Thryonomys* sp.) in these flies (Table S9); the mammal detection rate in the village was noticeably lower in these preliminary results than typical detection rates from flies captured in the forest (9% vs. 20-40%). While these results may suggest that flies do leave the forest, it is also possible that these flies were exposed to the larger mammal species' DNA through contact with bushmeat in the villages; both duikers and colobines are hunted frequently in the region, and the two rodent species detected are often found in human habitats (with cane rats even being bred for food). Further research, such as a mark recapture experiment at the forest edge, is needed to conclusively determine whether flies move between human and wildlife populations. Such research will be particularly relevant as expanding human populations come into increasingly close contact with non-human primate populations and their associated flies. Increasing evidence suggests that human (Köndgen et al., 2008) and domestic animal pathogens (Dobson et al., 1996) can spill-over or spill-back into wildlife populations and cause major population declines. It will thus also be important to understand whether synanthropic flies can move pathogens from human and domestic

571 animal populations into wildlife populations.

572

573 The DNA found in flies has been shown to be a promising tool for monitoring biodiversity (Calvignac -

574 Spencer et al., 2013; Lee et al., 2015; Schubert et al., 2015); where it is usually picked up by flies was not
575 investigated until now. Since many flies captured in the mangabey group tested positive for mangabey DNA
576 during a time when none of the habituated individuals had died, it is clear that flies were exposed to
577 mangabey DNA through sources other than carcasses. For those flies captured on feces, the feces itself
578 represents a likely source of mangabey DNA, but we also found mangabey DNA in flies captured with the
579 synthetic carcass bait. As discussed above, the fly species present appeared to be attracted to both the
580 synthetic carcass baits and feces, suggesting exposure to feces might be another source of exposure to
581 mangabey DNA for flies captured using synthetic carcass baits, though landing on a host and direct contact
582 with their fluids is possible as well. Considering that these mangabeys usually defecate several times a day
583 and can live for at least a decade, the amount of fecal biomass generated by a mangabey far outweighs the
584 weight of the carcass it leaves behind – given that flies feeding on feces contain enough mangabey DNA to
585 be detected with our molecular tools, perhaps only a minor proportion of randomly caught flies that contain
586 mammalian DNA acquired it from carcasses. Vertebrate fecal metabolites (urobilinoids) detected in adult
587 blow fly guts represent a potential means to identify when mammalian DNA came from contact with feces,
588 which might help future research differentiate the source of mammalian DNA found in flies (Owings et al.,
589 2018). Despite the potential rarity of carcass DNA in flies, our results certainly lend further support for such
590 an approach for monitoring bacterial pathogens (Hoffmann et al., 2017; Knauf et al., 2016).

591

592 The finding of these two bacterial pathogens in only 103 flies captured in this primate social group, suggests
593 that flies represent a cost effective and comparatively safe tool for pathogen monitoring. Sampling cadavers
594 or anesthetizing animals to detect pathogens in wildlife requires extensive training and resources and poses

595 a risk to the animals. A number of other highly infectious pathogens circulate in these ecosystems (e.g.,
596 monkeypox virus; 79, Ebola virus; 80), making the collection of necropsy samples a dangerous task best
597 left for trained experts; an untrained biologist attempting to collect necropsy samples in Taï National Park
598 was infected with a new strain of Ebola virus (Le Guenno et al., 1995). Thus, sampling flies may prove a
599 safe and useful tool for allowing a broader geographical and temporal screening to understand the
600 distribution of these and other pathogens in wildlife populations. We encourage researchers to use caution
601 while trapping flies (e.g., wearing gloves, disinfecting hands after work, disinfecting trapping materials
602 regularly) to minimize the potential risk of pathogen exposure, with the additional by-product that this will
603 help avoid contaminating flies with human DNA.
604
605 Collectively, our results suggest that attraction of flies might represent a previously underappreciated cost
606 to forming social groups. Further studies are needed to confirm whether variation in species social
607 organization (solitary vs. group-forming) and behavior (terrestrial vs. arboreal, group sizes) influences fly
608 density and whether such associations exist with other animals and in other ecosystems. The chimpanzees
609 of Taï forest represent an ideal study system for this purpose: chimpanzee groups fission and fusion, which
610 provides a natural experiment to examine how group sizes influence vector exposure, though our sample
611 sizes were not yet sufficient to explore the impact of this variable on fly densities. In fact, it has been
612 hypothesized that the fission-fusion behaviour of chimpanzees actually evolved to mitigate disease risk
613 (Lehmann et al., 2007) and it will be interesting to examine whether smaller parties are indeed less exposed
614 to potential arthropod vectors, including flies. It will also be interesting to assess whether such fly
615 associations also exist in more mobile human hunter gatherer populations and whether aspects of hunter
616 gatherer behaviour, such as the repeated moving of camps or the use of latrines, might also serve to reduce
617 exposure to flies and ultimately disease risk. Finally, it will be exciting to explore whether mechanical
618 vectors like the flies examined here are attracted to social groups more generally and have contributed to
619 shaping the ecology and evolution of social mammals more broadly.

ACKNOWLEDGEMENTS

We thank the Ministère de l'Enseignement Supérieur et de la Recherche Scientifique and the Ministère de Eaux et Fôrests in Côte d'Ivoire, and the Office Ivoirien des Parcs et Réserves for permitting the study. We are grateful to the Centre Suisse de Recherches Scientifiques en Côte d'Ivoire and the staff members of the Taï Chimpanzee Project for their support. We are indebted to the Max Planck Society providing core funding for the Taï Chimpanzee Project since 1997. For assistance in the field we thank Vera Leinert, Bekah Myers, Sylvain Lemoine, and the long-term mangabey field assistants Simon T. Kannieu, Daniel Bouin, and Gnimion Florent. For helpful R scripts used for plotting Figure 2 we thank Roger Mundry. For assistance culturing *Bcbva* under biosafety level 3 conditions we thank Silke Klee, Ursula Thiesen and Sabine Howaldt. This article represents work completed in part during the doctoral dissertation of J.F.G. and benefited greatly from the input of his advisor, Jonathan Davies, and his supervision committee, David Marcogliese, Charles Nunn, and Louis Lefebvre

FUNDING

This study was supported in part by the DFG Research Group "Sociality and Health in Primates" (FOR2136). JFG was supported by the Canadian Institutes of Health Research's Strategic Training Initiative in Health Research's Systems Biology Training Program, an NSERC Vanier Canada Graduate Scholarship (CGS), a long-term Research Grant from the German Academic Exchange Service (DAAD-91525837-57048249), and also by the DAAD with funds from the German Federal Ministry of Education and Research (BMBF) and the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme (FP7/2007-2013) under REA grant agreement n° 605728 (P.R.I.M.E. – Postdoctoral

Researchers International Mobility Experience). Core-funding for the Taï Chimpanzee Project is provided by the Max Planck Society.

ETHICS

Fly specimens were collected in accordance with the national laws of the Ivory Coast. The protocols regarding work with the wild primates followed in this study adhered to the American Society of Primatologists principles for the ethical treatment of primates. Appropriate ethical approval and licenses were obtained in collaboration with the Ministry of the Environment and Forests, the Ministry of Research, the directorship of the Taï National Park, and the Centre Suisse de Recherches Scientifiques en Côte d'Ivoire in Abidjan.

DATA ACCESSIBILITY

Next generation sequencing reads for constructing the *Bc*bv*a* genomes are available through the Short Read Archive (SRA) with the BioProject ID PRJNA545191. All additional DNA sequences generated as part of this analysis have be uploaded Dryad as an assignment to particular organism is always uncertain or redundant with existing sequences. All other raw data are available in the manuscript or in the supplementary material.

AUTHOR CONTRIBUTIONS

JFG, AD, JMT, SCS, and FHL conceived and designed the study with valuable input and discussions with all of the co-authors. JFG, AD, and JMT collected data in Taï National Park with support from AM and RMW who work with the non-human primates in this forest. JFG, AD, CH, BM, and AS conducted experiments in the lab. JFG and SCS conducted data analysis. JFG, SCS and FHL drafted the manuscript with subsequent input from all of the co-authors. All authors provided critical revision of the article and gave final approval to the version submitted for publication.

COMPETING INTERESTS

We have no competing interests.

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TABLES AND FIGURES

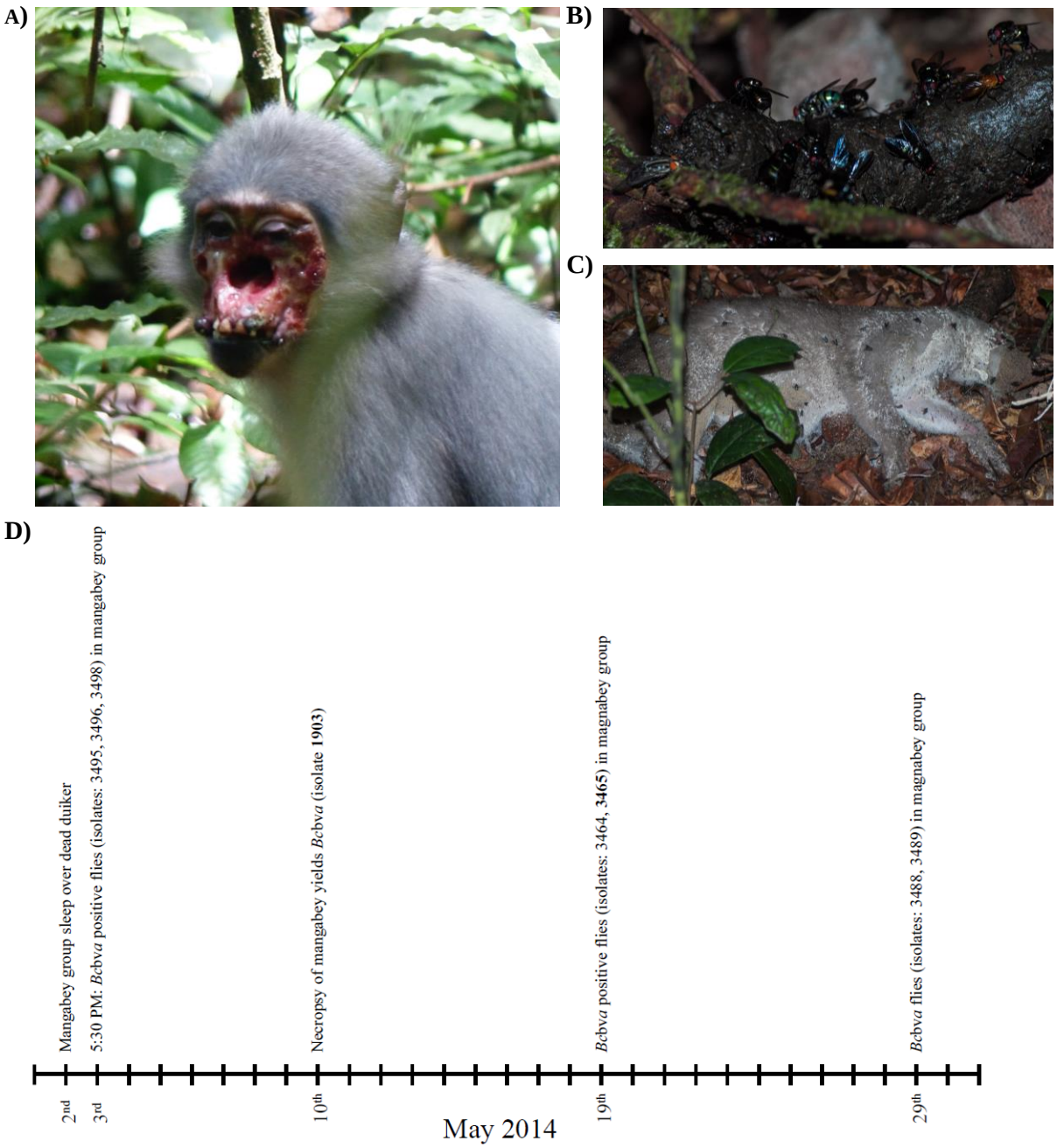

Figure 1: A) An adult mangabey in the study group with a severe *Treponema pallidum pertenue* infection showing extensive facial tissue destruction, including damage to bone and cartilage, and a poor general condition. **B)** Flies feeding on mangabey feces. **C)** A duiker carcass in TNP covered in flies and their larva. **D)** Schematic showing the timing of *Bc*bv*a* isolate collection: the fly isolate with only 1 variant position

with the dead mangabey isolate, which are thus either epidemiologically linked or stemmed from the same source, are highlighted in bold.

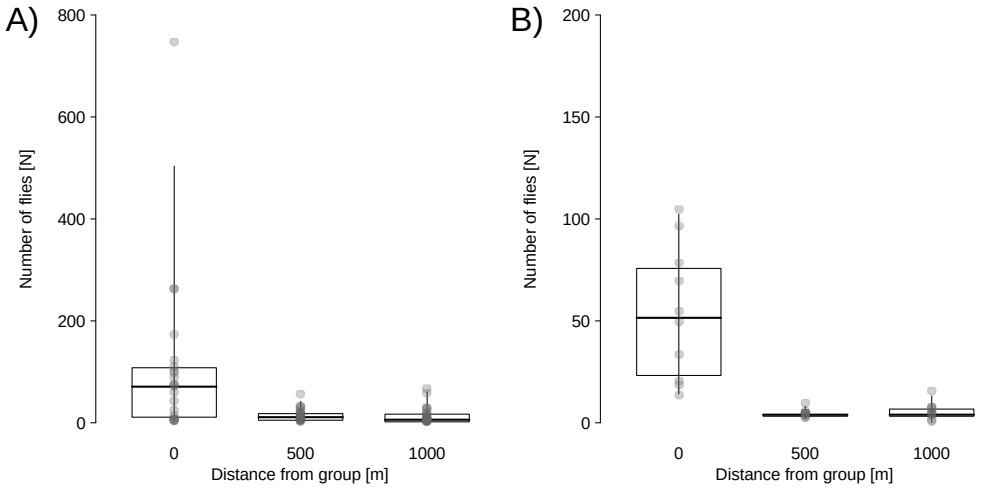

Figure 2: The number of flies captured at traps set at different distances from the mangabey social group using: A) synthetic carcass baited traps and B) feces baited traps. The middle horizontal line represents the median while the rectangle shows the quartiles and the vertical line represents the 2.5 and 97.5% percentiles and each circle indicates the number of flies caught in a particular trap.

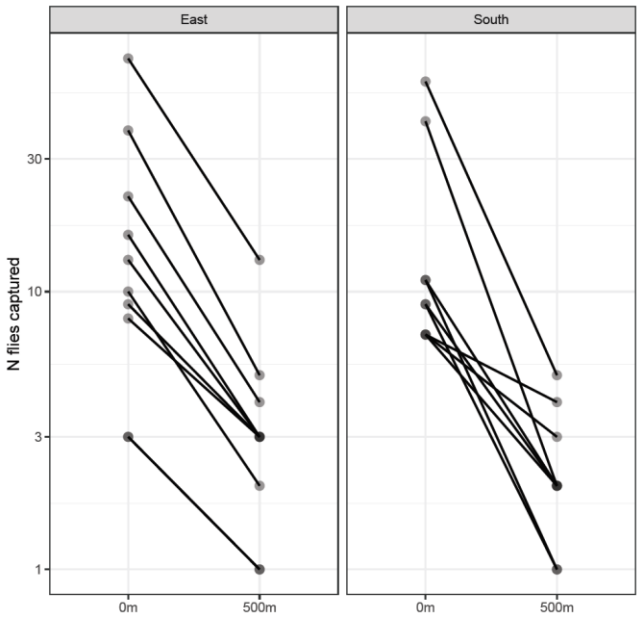

Figure 3: The number of flies captured with traps feces baited traps set within and 500m away from two neighboring chimpanzee communities. Lines connect paired traps set consecutively on the same day. The Y-axis is shown using a log scale.

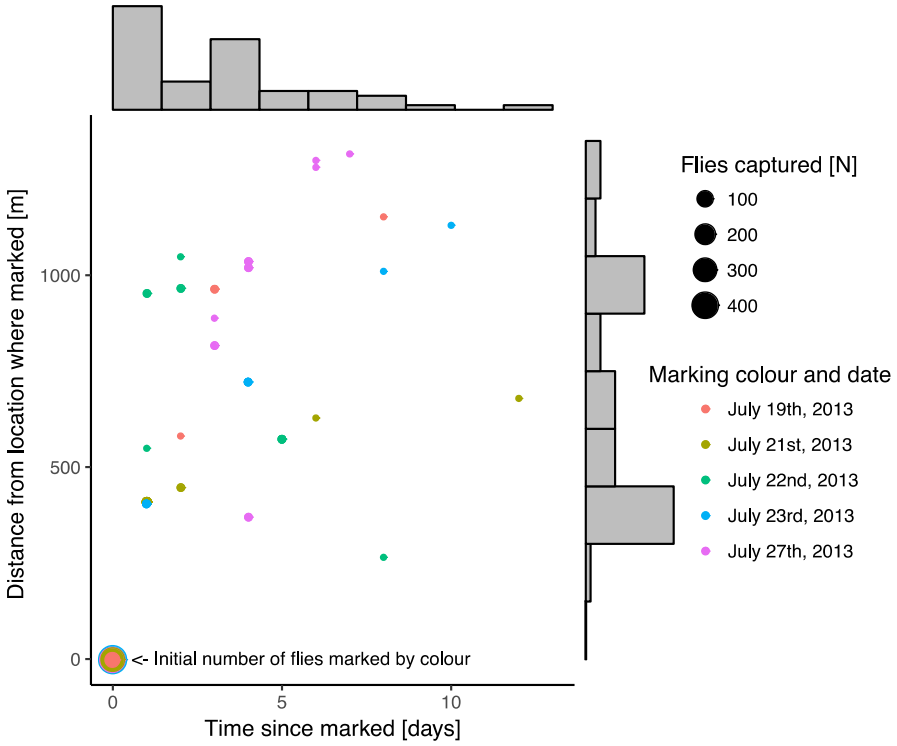

Figure 4: Scatter plot of flies recaptured in the sooty mangabey group indicating the distance from the location where they were initially marked and the number of days that elapsed between marking and recapturing. The size of the points is proportional to the number of flies recaptured or marked. The marginal histograms indicate the distribution of the distances from the location where marked and the time that elapsed between when they were marked and recaptured, and do not include the initial flies marked. In the lower left corner of the plot, the number of flies originally marked in each colour is indicated with overlapping circles.

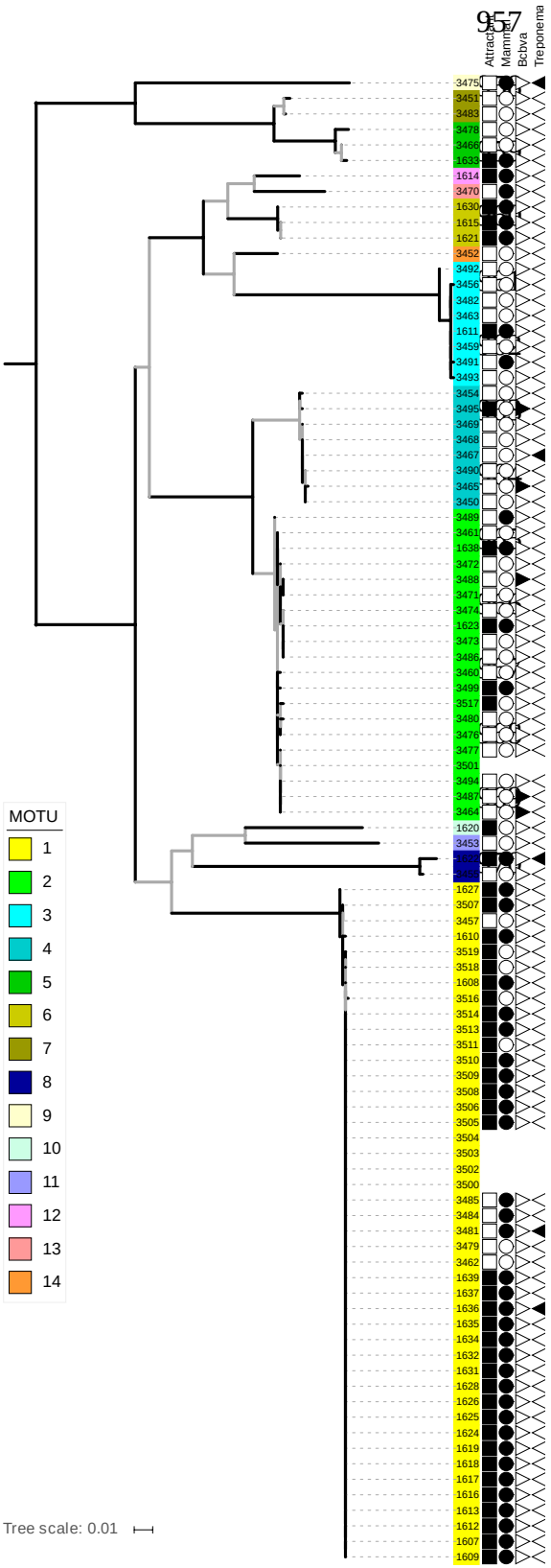

Figure 5: A maximum likelihood phylogeny generated using the CO1 sequences from flies captured using either feces (filled black square) or synthetic carcass (white square) as an attractant. Branches that received less than 0.95 Shimodaira–Hasegawa approximate likelihood ratio test (SH-like aLRT) support are indicated in grey. Numbers at the tips of the branches indicate the unique fly identification number. In total, 14 MOTU were identified with bPTP and each fly identification number is colored based on its MOTU. MOTUs were numbered from the most to the least abundant. Flies that contained mammal DNA are shown with a black circle, while those that contained *Bcbva* or *Treponema pallidum* DNA are indicated with a black triangle in the respective column.

Table 1: GLMM results for synthetic carcass baited traps and feces baited traps.

Synthetic carcass baited traps

Term	Estimate	SE	Lower CL	Upper CL	Z-value	P-value
Intercept - Within group	2.996	1.443	0.114	5.909	(1)	(1)
500m from group	-1.611	0.411	-2.464	-0.839	-3.920	<0.001
1000m from group	-1.880	0.419	-2.732	-1.0852	-4.486	<0.001
ID of capturer: JFG	0.129	0.337	-0.510	0.780	0.382	0.703
Hour trap was set	0.056	0.109	-0.164	0.276	0.510	0.610

Feces baited traps

Term	Estimate	SE	Lower CL	Upper CL	Z-value	P-value
Intercept - Within group	3.307	1.067	1.117	5.709	(1)	(1)
500m from group	-2.481	0.308	-3.104	-1.969	-8.056	<0.001
1000m from group	-2.337	0.304	-2.951	-1.829	-7.693	<0.001
ID of capturer: JMT	-0.0309	0.261	-0.581	0.471	-0.118	0.906
Hour trap was set	0.0392	0.083	-0.144	0.213	0.470	0.638

(1)Not shown because of not having a meaningful interpretation.

Table 2: Summary of pathogen screening for flies captured in the mangabey group.

	Treponema pallidum		*Bcbva*			
	% pos. (N pos. / N tested)		% pos. (N pos. / N tested)			
Trap type	polA seq.	cfpA seq.	*PA qPCR*	*CapB qPCR*	*Island IV qPCR*	*Pos. in all three assays*
Feces	5.2% (3/58)	33.3% (1/3)	8.6% (5/58)	50.0% (4/8)	42.9% (3/7)	5.2% (3/58)
Synthetic carcass	8.6% (3/45)	0.0% (0/6)	15.6% (7/45)	33.3% (4/12)	41.7% (5/12)	8.9% 4/45)

Table 3: Details regarding fly MOTUs.

MOTU	Tentative taxonomic assignment based on BLAST	Number of flies	MOTU support
1	Calyptratae: Ostroidea: Muscidae	44	0.84
2	Calyptratae: Ostroidea: Calliphoridae: *Chrysomya*	20	0.89
3	Calyptratae: Ostroidea: Sarcophagidae: *Sarcophaga*	8	0.46
4	Calyptratae: Ostroidea: Calliphoridae: *Chrysomya putoria*	8	0.88
5	Calyptratae	3	0.69
6	Calyptratae: Ostroidea: Calliphoridae	3	0.88
7	Calyptratae	2	0.98
8	Calyptratae	2	0.71
9	Calyptratae: Ostroidea	1	1.00
10	Calyptratae	1	1.00
11	Calyptratae	1	1.00
12	Calyptratae: Ostroidea: Calliphoridae	1	1.00
13	Calyptratae: Ostroidea: Calliphoridae	1	1.00
14	Calyptratae: Ostroidea: Calliphoridae: *Hemigymnochaeta*	1	1.00

Table 4: Results of Bayesian Tip-association Significance testing (BaTS) of bait type, and mammal, *Treponema pallidum*, or *Bc*bv*a* detection on the fly phylogeny (Figure 5). Only flies for which data was available for each of the four parameters were included in this analysis (98 flies).

Parameter	Statistic	Observed mean	Lower 95% CI	Upper 95% CI	Null mean	Lower 95% CI	Upper 95% CI	Significance (P-value)
Bait type	AI	3.67	2.92	4.38	5.32	4.62	6.06	**<0.001**
	PS	17.62	16.00	18.00	30.70	28.07	33.22	**<0.001**
	MC (feces)	7.67	4.00	13.00	3.83	3.16	5.74	**0.020**
	MC (synthetic)	6.17	5.00	7.00	3.48	2.88	4.37	**0.020**
Mammal detection	AI	3.21	2.40	4.01	5.22	4.51	5.90	**<0.001**
	PS	18.10	16.00	19.00	30.47	27.83	32.86	**<0.001**
	MC (yes)	7.00	4.00	12.00	3.91	3.12	5.87	**0.030**
	MC (no)	8.03	8.00	8.00	3.63	2.92	5.87	**0.020**
T. pallidum detection	AI	0.93	0.55	1.27	1.08	0.91	1.25	0.110
	PS	4.98	5.00	5.00	4.94	4.80	5.00	1.000
	MC (yes)	1.02	1.00	1.00	1.06	1.00	1.20	1.000
	MC (no)	19.69	19.00	24.00	19.57	14.16	37.91	0.880
*Bc*bv*a* detection	AI	0.92	0.43	1.37	1.09	0.80	1.26	0.150
	PS	4.69	4.00	5.00	4.92	4.71	5.00	1.000
	MC (yes)	1.29	1.00	2.00	1.08	1.00	1.27	1.000
	MC (no)	45.89	40.00	58.00	19.90	12.89	40.17	**0.030**

AI - association index; PS - Fitch parsimony score; MC - monophyletic clade statistic indicating the maximum observed exclusive single-state clade size.

SUPPLEMENTARY DATA

Figure S1: A schematic detailing how the feces baited traps were set up.

Table S1: Summary of shotgun sequencing of *Bc*bv*a* isolates from flies and the dead mangabey.

Table S2: The number of flies captured at each trapping event at different distances from the mangabey group using synthetic carcass baited traps.

Table S3: The number of flies captured at each trapping event at different distances from the mangabey group using feces baited traps.

Table S4: The number of flies captured at each trapping event with and outside the two neighboring chimpanzee communities using feces baited traps.

Table S5: Full results from the mark recapture experiment.

Table S6: Full results for pathogen screening of flies captured on feces and using synthetic baits along with results of mammal DNA detection in flies and fly species determination.

Table S7: Variant positions confidently identified in the dead mangabey *Bc*bv*a* isolate (1903) and whether or not they were shared with each of the 7 fly *Bc*bv*a* isolates.

Table S8: Variant positions confidently identified in the fly *Bc*bv*a* isolates and whether or not they were shared with the dead mangabey isolate (1903).

Table S9: Results of pathogen screening of flies captured on in villages near Taï National Park captured using synthetic baits, along with results of mammal DNA detected in flies and fly species determination.