SOCIAL AND ECOLOGICAL DRIVERS OF PATHOGEN TRANSMISSION DYNAMICS IN EAST AFRICAN GREAT APES

by

Julie Lynn Rushmore

(Under the Direction of Sonia Altizer)

Abstract

Infectious diseases have threatened the health of Africa's endangered great apes. Work on pathogen dynamics in humans demonstrates that pathogen transmission can increase with social and mating contacts, yet few studies have examined the role of host behavior in wildlife pathogen spread. Further, despite promiscuous mating behavior among African apes (i.e., chimpanzees, bonobos and to a lesser extent gorillas), little is known about the prevalence or impact of sexually transmitted diseases (STDs) on this primate group. To better understand the social and ecological drivers of pathogen transmission dynamics in wild apes, I used a combination of field, molecular, and mathematical modeling techniques to 1) assess how temporal contact heterogeneity affects pathogen dynamics and control, and 2) examine the diversity and prevalence of potential STDs. To address goal 1, I collected nine months of behavioral association data from a wild chimpanzee community in Kibale Forest, Uganda, and I used these data to build monthly chimpanzee contact networks. I then used a combination of network analysis and epidemiological modeling to simulate pathogen spread on networks for a range of pathogen types. To explore optimal pathogen control strategies, I identified risk groups of individuals most likely to initiate large outbreaks and

compared model simulations of network-based vaccination strategies that targeted these risk groups. To address goal 2, I collected and screened samples from wild and sanctuary chimpanzees and gorillas for putative STDs, compared infection status against ecological factors, and used sequence analysis to better characterize positive samples. Overall, this work represents a multi-disciplinary approach to understand social and ecological factors affecting pathogen transmission in East African apes and provides crucial information for developing management strategies to protect endangered apes from current and future disease threats.

INDEX WORDS: disease control, epidemiology, *Gorilla beringei*, *Pan troglodytes*, sexually transmitted disease, social network analysis, wildlife conservation

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Dedication

To the Kanyawara chimpanzees. I feel very fortunate to have spent this time with them.

Acknowledgments

Studying great apes in the wild and contributing thoughtful research to primate conservation efforts has been a lifelong dream of mine. I am incredibly grateful for this experience and for the generous help that I received from many people along the way.

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Chapter 1

INTRODUCTION AND LITERATURE REVIEW

A key objective of epidemiology is to identify host traits that affect pathogen transmission dynamics (Anderson and May 1991; Keeling and Rohani 2008). Because many directly transmitted pathogens spread through social contacts, highly social individuals are expected to have a greater infection probability than less social individuals, and might also contribute disproportionately to pathogen spread (Altizer et al. 2003). Thus, social behaviors that affect the frequency and duration of contacts, and the underlying ecological factors that shape social interactions, can play a prominent role in determining patterns of pathogen transmission (Altizer et al. 2006, 2003). The major aim of this dissertation is to examine social and ecological drivers of pathogen transmission in an endangered wildlife host system.

Host social organization can affect individual infection risk in several ways. Specifically, host parasite burden often increases with social group size (Hoogland 1979; Poulin 1995; Wilkinson 1985) and host density (Nunn et al. 2000; Packer et al. 1999). Similarly, species with promiscuous mating systems are expected to harbor more sexually transmitted diseases (STDs) than species with monogamous mating systems (Nunn and Altizer 2004), and several

studies have demonstrated that host immune defenses (a proxy for pathogen risk) increase with promiscuity (Nunn et al. 2003; Nunn 2002; Nunn et al. 2000). Further, within a species or population, individual traits (such as age, sex, or dominance rank) can greatly affect individual exposure to pathogens through variation in contact rates. For example, Caillaud et al. (2006) demonstrated that during a 2003-2004 Ebola outbreak in Congo, gorillas living in social groups (i.e., dominant adult males, adult females, and juveniles) were more than twice as likely to die of Ebola than bachelor males not living in social groups. These differences in mortality were largely attributed group-living gorillas having higher contact rates and thus increased exposure to infected individuals.

Ecological and seasonal factors can influence host behavior, thus affecting frequency and duration of contacts (Altizer et al. 2006). In humans, childhood diseases such as measles are regulated in part by the academic calendar year, in which contact rates and infection levels rise when school is in session and fall and when school is out of session (Fine and Clarkson 1982; Finkenstädt and Grenfell 2000). Similarly, patterns of host aggregation in wildlife species that are driven by mating events or resource availability are expected to alter contact rates and thus pathogen transmission dynamics (Altizer et al. 2006; Newton-Fisher et al. 2000). For example, phocine distemper virus outbreaks in seals have been shown to coincide with seasonal haul-outs, in which seals form densely packed mating groups on land (Swinton et al. 1998). Annual outbreaks of house finch conjunctivitis similarly coincide with the fall months when susceptible juveniles and adults aggregate and intermingle in flocks at backyard bird feeders (Altizer et al. 2004). Elucidating the social and ecological processes that affect pathogen transmission dynamics will help predict and control outbreaks in both human and animal systems.

To characterize patterns of inter-individual contacts, populations can be represented as networks, in which nodes depict individuals (or groups of individuals) and edges represent contacts allowing for pathogen transmission (Newman 2010). Thus, social network analysis

offers an effective approach to mathematically formalize transmission pathways and host contact variation (Newman 2010). Further, network analysis can identify superspreaders, which are individuals with disproportionately high contact rates that could be targeted for control efforts (Lloyd-Smith et al. 2005). Studies of human contact networks have advanced scientific understanding of transmission dynamics for directly transmitted pathogens such as SIV and HIV (Anderson et al. 1990; Lloyd-Smith et al. 2005; Meyers et al. 2005). Modeling studies in human populations have demonstrated that targeting the most connected individuals for pathogen control strategies such as vaccination can be much more effective than random control (Lloyd-Smith et al. 2005; Salathé et al. 2010). However, because contact networks are more difficult to define for animal populations than human populations (largely because animals cannot self-report their contacts the way that humans can), network analysis is rarely used to investigate the epidemiology and control of wildlife diseases (Craft and Caillaud 2011).

Owing to their complex and often well-studied social systems, wild primates and especially African apes (e.g., chimpanzees, *Pan troglodytes*; bonobos, *Pan paniscus*; and to lesser extent gorillas, *Gorilla beringei*) could help bridge this gap. In particular, habituated great ape communities, which have been the focus of several long-term research projects (e.g., Wrangham and Ross 2008), offer a unique opportunity to conduct community-wide observations of fine-scale individual contacts. Notably, because apes in these communities are habituated to human observers, there is no need for the expensive technologies or invasive procedures typically required for collecting detailed observational data via tracking devices (e.g., radio-tracking devices and proximity-logging collars: Cross et al. 2004; Hamede et al. 2009). Further, all great apes are listed by the IUCN as endangered species (IUCN 2012), and pathogens are a key cause of ape population declines (Ryan and Walsh 2011). Specifically, outbreaks of Ebola and respiratory infections have caused dramatic declines in several gorilla and chimpanzee populations (Bermejo et al. 2006; Kaur et al. 2008; Köndgen et al.

2008). Importantly, African apes are highly social animals that frequently interact with group members while foraging, playing, and grooming (Goodall 1986; Kano 1992; Schaller 1963). Further, chimpanzees and bonobos are also considered to be highly promiscuous (Goodall 1986; Kano 1992), and mating contacts can provide key transfer routes for STDs and non-STDs alike (Nunn and Altizer 2006; Nunn et al. 2000).

In a comprehensive review article, Lockhart et al. (1996) determined that over 200 STDs have been reported among wild animal species. However, wild apes have not yet been comprehensively studied for STDs, perhaps because STDs rarely lead to visible clinical signs (Holmes et al. 2008; Lockhart et al. 1996). In humans, STDs can cause sterility and infant mortality (Ntozi 2002). STDs have also been reported to cause declines in some wildlife populations (Augustine 1998). Because STDs can go undetected for long periods and might lower population recruitment in small host populations (Lockhart et al. 1996), research aimed at investigating naturally occurring ape STDs could inform efforts to manage and conserve wild ape populations.

To better understand how social and ecological drivers affect pathogen transmission dynamics in East African apes, this dissertation undertook two key goals: i) integrate field behavioral data with network-based epidemiological models to simulate the transmission and control of pathogens within a wild ape community (Chapters 2 and 3), and ii) use field- and sanctuary-collected samples to quantify the occurrence of STDs in East African apes (Chapter 4). To address the first goal, I observed behavioral associations in a habituated wild chimpanzee community at Kanyawara, Kibale National Park in Uganda. In Chapter 2, I used these field data to construct monthly party (i.e., group) and close-contact (i.e., $\leq$ 5m) association networks over a period of nine months. Using a combination of network analysis and Bayesian approaches, I assessed how contact patterns change over time in relation to estrous events and seasonal changes in fruit availability. To determine which individuals are likely to generate large outbreaks, I used permutation tests and examined how individual

traits (e.g., age, sex, dominance status) affect an individual's connectivity and position in the monthly networks.

In Chapter 3, I combined the chimpanzee behavioral association data with epidemiological network models to design and evaluate disease intervention strategies. Specifically, I simulated outbreaks on monthly contact networks parameterized with association data from the Kanyawara chimpanzee community to ask how final outbreak size depends on the network position of the index case, outbreak timing, and pathogen infectiousness. I then used permutation tests to determine traits associated with individuals most likely to initiate large epidemics and to identify risk groups that could be targeted for pathogen control. Lastly, I simulated vaccination on the observed chimpanzee networks to evaluate the effectiveness of network-based control efforts as compared to randomly applied control efforts.

To address the second project goal, I collaborated with the Kibale Chimpanzee Project (KCP), the Mountain Gorilla Veterinary Project (MGVP), and the Chimpanzee Sanctuary and Wildlife Conservation Trust (CSWCT) to collect biological samples from East African chimpanzees and gorillas in the wild and in African sanctuaries. In Chapter 4, I used these samples to screen East African apes for putative STDs from four major groups. Using generalized linear models, I examined the social and ecological factors associated with individual infection status. I also used molecular techniques to build a phylogeny of one pathogen group using sequence data from positive samples. Ultimately, this dissertation lends support for the effects of social and ecological variables on pathogen transmission dynamics in a wildlife species. Further, this work shows that behavioral and ecological factors can play an important role in forecasting outbreaks and designing vaccination programs for endangered wildlife.

Chapter 2

SOCIAL NETWORK ANALYSIS OF WILD CHIMPANZEES PROVIDES INSIGHTS FOR PREDICTING INFECTIOUS DISEASE RISK

J. Rushmore, D. Caillaud, L. Matamba, R. M. Stumpf, S. P. Borgatti, and S. Altizer. Accepted by *Journal of Animal Ecology*. Reprinted here with permission of the publisher.

2.1 Abstract

Heterogeneity in host associations can alter pathogen transmission and strategies for control. Great apes are highly social and endangered animals that have experienced substantial population declines from directly transmitted pathogens; as such, network approaches to quantify contact heterogeneity could be crucially important for predicting infection probability and outbreak size following pathogen introduction, especially owing to challenges in collecting real-time infection data for endangered wildlife. We present here the first study using network analysis to quantify contact heterogeneity in wild apes, with applications for predicting community-wide infectious disease risk. Specifically, within a wild chimpanzee community, we ask how associations between individuals vary over time, and we identify traits of highly connected individuals that might contribute disproportionately to pathogen spread. We used field observations of behavioral encounters in a habituated wild chimpanzee community in Kibale Forest, Uganda to construct party-level (i.e., subgroup) and close-contact (i.e., $\leq$ 5m) association networks over a nine-month period. Network analysis revealed that networks were highly dynamic over time. In particular, estrous events significantly increased pairwise party associations, suggesting that community-wide disease outbreaks should be more likely to occur when many females are in estrus. Bayesian mixed-effects models and permutation tests identified traits of chimpanzees that were highly connected within the network. Individuals with large families (i.e., mothers and their juveniles) that range in the core of the community territory and to a lesser extent high-ranking males were central to association networks, and thus represent the most important individuals to target for disease intervention strategies. Overall, we show striking temporal variation in network structure and traits that predict association patterns in a wild chimpanzee community. These empirically-derived networks can inform dynamic models of pathogen transmission and have practical applications for infectious disease management of endangered wildlife species.

Key words: association patterns, infectious disease dynamics, Pan troglodytes, wildlife conservation, pathogen control

2.2 Introduction

Many pathogens spread through host populations via social interactions (Altizer et al. 2003); thus, knowledge of a community's social system and contact structure can provide crucial information for predicting infectious disease outbreaks (e.g., Drewe 2010; Griffin and Nunn 2012; Nunn et al. 2008). Inter-individual contacts that lead to pathogen transmission can be represented using networks, where each node represents an individual, and edges between nodes represent interactions that allow for pathogen transmission. Contact networks for humans and animals are often heterogeneous (e.g., Lusseau 2003; Schneeberger et al. 2004; Wey and Blumstein 2010), which violates the common assumption of many basic epidemiological models that contacts are random and individuals are well mixed (Anderson and May 1991). Network analysis provides a feasible (albeit data intensive) approach to mathematically formalize transmission pathways and host contact variation (Newman 2010). Further, network analysis can identify potential superspreaders, individuals with disproportionately high contact levels, that could be targeted for vaccination, treatment, or isolation (Lloyd-Smith et al. 2005). Studies of human contact networks often detect heterogeneity and the presence of superspreaders, which has been extremely influential in our understanding of transmission dynamics for SARS and HIV/AIDS (Anderson et al. 1990; Lloyd-Smith et al. 2005; Meyers et al. 2005). Superspreaders have also been identified in a few wildlife populations (e.g., possums and deer mice: Clay et al. 2009; Porphyre et al. 2008); however, network analysis is rarely used to investigate the epidemiology and control of wildlife diseases (Craft and Caillaud 2011). Here we present the first study to analyze empirical wild chimpanzee contact networks within a framework of predicting implications for infectious disease risk.

Endangered wild ape populations have recently experienced outbreaks of Ebola, measles and respiratory viruses, making infectious disease a major threat to their survival (Ryan and Walsh 2011), in part owing to the risk of pathogen spillover from humans to wild apes (Kaur et al. 2008; Köndgen et al. 2008). Thus, given the push to habituate wild apes for tourism across more than 15 African sites (Muehlenbein and Ancrenaz 2009), infectious disease risks for apes will likely continue or escalate. Respiratory diseases in particular have resulted in outbreaks with up to 25% community-level mortality at several long-term chimpanzee research sites (Ryan and Walsh 2011). With low birth rates and late reproductive maturity, ape populations can take decades to recover in size after an outbreak. For example, using mathematical models and a range of parameters derived from published ape epidemics (i.e., mortality rates of 4 – 25%), Ryan and Walsh (2011) estimated that a mountain gorilla population would require 5 – 32 years to recover following an outbreak of respiratory disease. In accordance with these predictions, a Tanzanian chimpanzee community took 15 years to return to its pre-epidemic population size after a 1987 respiratory disease outbreak (Williams et al. 2008).

In addition to the detrimental impact that pathogens can have on endangered apes, obtaining real-time infection data for wildlife is notoriously difficult. Collecting biological samples often requires risky interventions including darting and possibly anesthetizing immune-challenged individuals. Furthermore, the speed with which respiratory pathogens typically spread through ape communities (e.g., with a duration of roughly two weeks to two months: Hanamura et al. 2008; Köndgen et al. 2010; Williams et al. 2008) can limit researchers' abilities to collect comprehensive health data during an outbreak. Given these challenges, parameterizing realistic epidemiological models with empirical association data (Davis et al. 2008; Hamede et al. 2011) is essential for developing strategies to reduce the risk and impact of infectious diseases. An underlying assumption of these models is that network edges represent possible pathogen transmission routes. Indeed, while relatively few

wildlife studies have both host infection and host association data, there is a growing body of evidence that wildlife social networks strongly predict individual infection status (Bull et al. 2012; Leu et al. 2010; Otterstatter and Thomson 2007) and that highly connected individuals tend to have greater parasite burdens than less connected individuals (Corner et al. 2003; Godfrey et al. 2009; Leu et al. 2010, but see: Otterstatter and Thomson, 2007).

Great ape societies are highly structured and complex. Chimpanzees in particular live in permanent social groups termed communities, and have a fission-fusion social structure, whereby individuals within the community frequently break off into subgroups, called parties, that vary in size and composition (Goodall 1986). A chimpanzee mother and her offspring travel together in a family unit, and sociality can vary greatly among adult females (Boesch and Boesch-Achermann 2000). In fact, Goodall (1986) noted that eastern chimpanzee females ranging in the core of the community's territory encountered other individuals on a daily or weekly basis, whereas females ranging on the periphery of the territory might encounter community members only a few times per year. Compared to females, males follow a linear dominance hierarchy (Muller and Wrangham 2004) and tend to be more gregarious (Gilby and Wrangham 2008). Additionally, other studies showed that party size tends to increase when females are in estrus or when ripe fruits are available (e.g., Anderson et al. 2002; Itoh and Nishida 2007; Wrangham 2000).

In this study, we use network analysis to examine association patterns among individuals in a community of wild chimpanzees at Kibale National Park, Uganda. In particular, we quantify how association patterns that represent potential pathogen transmission routes vary over time, in response to factors such as fruit availability or the number of estrous females. We also examine individual traits that contribute to high levels of association, and predict that high-ranking males and estrous females will have disproportionately high levels of association with community members owing to increased rates of grooming and mating (e.g., Emery Thompson and Wrangham 2008; Goodall 1986). Importantly, investigating the

dynamics and drivers of contact variation in wild apes is a necessary step for simulating pathogen spread and evaluating the success of much needed disease intervention strategies for this highly threatened primate clade.

2.3 Methods

Study site and population

We studied the habituated wild Kanyawara chimpanzee community at Kibale National Park (0 °34′N, 30 °21′E) in Uganda. The site is dominated by moist deciduous forest interspersed with secondary forest, grassland, and swamp (Chapman and Wrangham 1993). Weather data for the site were provided by C. Chapman. Further details on the ecology of Kibale are discussed in Struhsaker (1997). The Kanywara chimpanzee community occupies roughly 37.8 km^2 of forest (Wilson et al. 2001), and during the time of the study the community included 48 chimpanzees with 12 adult males (aged > 14), 14 adult females (aged > 13), 9 immature males and 6 immature females (aged between 5 – 14 and 5 – 13 respectively; hereafter referred to as juveniles), and 7 dependent offspring (aged ≤ 4). For additional information on the Kanyawara community, see Supporting Information (Appendix Text A.1.1).

Data collection

We collected data on chimpanzee association patterns over nine months between Dec 2009 – Aug 2010 for 4 – 6 days per week between 6:00am and 7:30pm. Each morning, we randomly selected a focal chimpanzee from a party (typically at a nest site) to follow for 10 hours. Every 15 min, we scanned the focal individual's party and recorded the identity of all party members based on individuals within a 50m radius, a common criterion for estimating chimpanzee party sizes (Clark and Wrangham 1994). As an index for assessing patterns of close

association within parties, at the same 15-min intervals, we also recorded pairs of individuals that were within 5m of each other, which is a measure that commonly contributes to identifying close associations among primates (e.g., Gilby and Wrangham 2008). We limited our focal follows and party composition data to chimpanzees greater than 4 years of age (i.e., excluding dependent offspring, which remain in close contact with their mothers); we also excluded two adult females and a juvenile male on the periphery of the community who were observed only twice during the study. Our total sample size was 37 individuals (12 adult males, 12 adult females, 7 juvenile males, and 6 juvenile females). We recorded days when parous females had maximal sexual swellings and noted ripe fruit species on which focal animals foraged.

Estimating association indices

We calculated monthly pairwise association indices between individuals at two spatial scales: *i*) party-level association indices were based on the frequency of monthly co-occurrence in the same party, and *ii*) close contact association indices (i.e., within-party and overall 5m-associations, described below) were based on the frequency with which two individuals were seen within 5m of each other during a given month. We examined associations at the party-level as a proxy for the transmission of pathogens spread by non-close contact (e.g., via fomites, aerosol transmission, or fecal-oral routes). To estimate party-level associations, we calculated a monthly 'twice weight index' (Cairns and Schwager 1987), hereafter referred to as a monthly party association index (PAI), from party membership scans. This parameter calculates the ratio of scans in which chimpanzees A and B were observed in the same party relative to the total number of scans in which either A or B was observed in any party as follows:

$$PAI_{AB} = \frac{S_{AB}}{S_A + S_B + S_{AB}} \tag{2.1}$$

where S_{AB} represents the number of scans where A and B were observed in the same party, S_A represents scans where A was observed in a party without B, and S_B represents scans where B was observed in a party without A. PAIs and subsequent indices described below could range from 0 (i.e., individuals in a pair were never observed associating in the given month) to 1 (i.e., individuals in a pair were observed to be associating during 100% of the observations for the given month).

Close-contact interactions were examined as a proxy for pathogens requiring direct contact or respiratory droplets to spread. As one close-contact measure, within-party association indices (WPAI) represent the proportion of scans in which chimpanzees A and B were observed within 5m of each other, given that they were within the same party:

$$WPAI_{AB} = \frac{S_{AB5}}{S_{AB}} \tag{2.2}$$

where S_{AB5} represents the number of scans where A and B were observed within 5m of each other. To examine which individuals were most central to the 5m-networks, we calculated an overall 5m-association index (5mAI), which incorporated the probabilities that individuals A and B would be both within the same party and within 5m of each other:

$$5mAI_{AB} = PAI_{AB} \cdot WPAI_{AB} \tag{2.3}$$

Thus, this index estimates the overall proportion of time that individuals A and B were within a 5m distance.

To examine host interactions at a temporal scale that reflects the transmission biology of real-world pathogens, we analyzed association patterns at both two-week and monthly intervals, as respiratory diseases common to chimpanzees and humans have infectious periods that range from a few days to one month (e.g., influenza: 2 – 3 days, measles: 6 – 7 days, chicken pox: 10 – 11 days, *Streptococcus spp.*: 14 – 30 days; Anderson and May 1991; Ekdahl et al. 1997) and published reports of wild chimpanzee respiratory illnesses suggest

that epidemic durations often range from roughly two weeks to two months (Hanamura et al. 2008; Köndgen et al. 2010; Williams et al. 2008). Because associations across both time steps were significantly correlated for both PAIs and 5mAIs (Appendix A: Table A.1), and other results were robust across both time steps, we present results for monthly associations in the main text (see Appendix A Tables A.2 and A.3 for two-week time step results).

Visualizing networks

We constructed monthly party and 5m-association networks in R version 2.15.1 (R Core Development Team 2010) with the igraph package version 0.5.5-4 (Csardi and Nepusz 2006). Party and 5m-network edges were weighted according to the monthly pairwise PAIs and 5mAIs respectively, such that pairs with higher association indices had thicker edges.

Individual trait data

In all analyses, we categorized chimpanzees based on their age, sex, dominance rank category, family size (Table A.4), and for pairwise analyses, whether two individuals were related to each other. Chimpanzee rank, based on dominance interactions for adult males, was categorized such that high-, medium-, and low-ranking adult males respectively occupied the rank categories of Male 1 (M1, $n = 5$), Male 2 (M2, $n = 4$), and Male 3 (M3, $n = 3$). By grouping individuals in this way, all males stayed within their respective rank categories throughout the study period, despite minor reshuffling in the linear hierarchy. Female chimpanzees rarely show dominance interactions; however, females occupying and foraging in the core area of the territory (at Kanyawara) tend to be higher ranking than those occupying the peripheral areas (Kahlenberg et al. 2008). Thus, we assigned core-area adult females and their juvenile offspring to rank categories Female 1 (F1, $n = 6$) and Juvenile 1 (J1, $n = 9$), and edge-ranging adult females and their offspring to Female 2 (F2, n

= 6) and Juvenile 2 (J2, $n = 4$). Additional details on rank categorization are in Appendix Text A.1.2 and Table A.4.

Lastly, we defined a family unit as a mother and her non-infant offspring, such that an individual's family size was the total number of non-infant chimpanzees in this family unit. In one unique case, a young adult male and his juvenile sibling were considered a family unit (Table A.4), as their mother was deceased. Chimpanzees who traveled without a family unit (e.g., adult males, females with infants only) were assigned a family size of one. We considered mother-offspring pairs and maternal siblings to be related, based on long-term records from the field site.

Monthly changes in network density

To compare PAIs and 5mAIs over time, we calculated monthly network density as the sum of the network's observed edge weights divided by the sum of the maximum possible edge weights (Hanneman and Riddle 2005). To examine how stable party and 5m-networks were over time, we assessed correlations between monthly association index matrices using a quadratic assignment procedure (see Appendix Text A.1.3 for details) in UCINET version 6.343 (Borgatti et al. 2002).

Analyses of pairwise associations

To examine how social factors (e.g., rank status) and ecological factors (e.g., fruit availability) affect temporal pairwise associations at party and 5m-levels, we fit two models (for PAI and WPAI data, respectively) to Bayesian logistic mixed-effects models using a Markov chain Monte Carlo (MCMC) framework. We tested for significant relationships between monthly pairwise associations and the following predictor variables: age (adult-adult, adult-juvenile, juvenile-juvenile), sex/estrus (i.e., pairwise combinations of males, non-estrous females, and

estrous females, *Note*: parous females were categorized as estrous during months in which they were observed to be in estrus; nulliparous females were never categorized as estrous), relatedness (related, unrelated), difference in family size (range: 0 – 3), and difference in rank category (scored as 1/0 where a pair in the same rank category scored a 0 and a pair in different ranks scored a 1). Because we expected mothers and their juveniles to associate frequently, for this analysis we collapsed the adult female and juvenile ranks into FJ1 (core-ranging individuals) and FJ2 (edge-ranging individuals).

We also included two key parameters that could affect associations over time. First, we included a parameter for the number of parous estrous females observed during each month, as males prefer mating with parous over nulliparous females (Muller et al. 2006). Additionally, research at some sites shows that increased fruit availability is linked to larger parties (e.g., Wrangham 2000). We did not have fruit abundance data; however, we included parameters for the monthly presence/absence of preferred ripe fruit species (*Mimusops bagshawei*, *Pseudospondias microcarpa*, *Uvariopsis congensis*: Wrangham et al. 1996) according to our focal data, as eating of preferred fruits is strongly associated with fruit availability for Kanyawara chimpanzees (Wrangham et al. 1991). We also included a parameter for the mean daily rainfall from two months prior, which we considered to be a proxy for current fruit availability.

To account for autocorrelation from repeated measures, we assessed model fit with random effects of chimpanzee ID, chimpanzee pair, and month. One difficulty with including a random effect for individual ID was that an individual could appear interchangeably as individual A or individual B in the observed pairwise associations described in equations 1 – 3. This interchangeability was due to the fact that the associations were not directed, meaning they did not have a specific 'sender' and 'receiver.' We resolved this issue by using the multi-membership modeling capabilities of the MCMCglmm package (Hadfield 2010) in R. Additional analysis details are in Appendix Text A.1.3; R code is available upon request.

Individual traits associated with network centrality

To identify individual traits associated with increased contact, we used UCINET to calculate three weighted network centrality measures for each chimpanzee: degree, eigenvector, and flow-betweenness. Weighted degree centrality (hereafter referred to as degree) for each node is the sum of the node's edge weights (Newman 2010). Eigenvector centrality is based on an individual's connectedness and the connectedness of an individual's associates, where an individual with high eigenvector centrality is connected to well-connected associates (Newman 2010). Lastly, flow-betweenness centrality is defined as the proportion of times an individual lies along the shortest path between pairs in the network (Freeman et al. 1991). Previous theoretical and empirical work in human and wildlife systems has shown that individuals with high degree, eigenvector, or flow-betweenness centrality are more likely to contract and transmit pathogens than individuals with low centrality (e.g., Corner et al. 2003; Salathé et al. 2010).

Using node-level permutation-based regressions, we fit individual centrality data in R with 30,000 permutations per test to investigate relationships between each centrality measure and the following predictor variables: rank, estrous-status, family size, continuous age, and sex (while controlling for month effects). We controlled for sampling effort across individuals by weighting the model variance structure according to the number of scans in which each individual was a focal subject. To account for comparisons of three centrality measures, we applied a Bonferroni correction and considered relationships where $P < 0.017$ (i.e., $P < 0.05/3$) to be significant. Age and sex were excluded from the final models because they were confounded with rank (which was already separated by age and sex groups), explained less than one percent of the variation (as determined by adjusted R^2), and were never significant after Bonferroni correction. Additional analysis details are in Appendix Text A.1.3.

2.4 Results

Association patterns and social network descriptions

On average, each chimpanzee was followed as a focal subject for 27.79 ($\pm$ 3.6) hours (Fig. A.1), comprising a total of 1,028 focal observation hours and 4,114 fifteen-minute scans for all individuals combined. Our analysis included 306,212 pairwise party associations and 14,673 pairwise 5m-associations over the nine month period. When averaged across months and individuals, randomly selected chimpanzee pairs were observed associating at the party-level approximately 26% of the time (mean PAI: 0.255, range: 0.0 – 1.0, SE: 0.003) and at the 5m-level 4% of the time (mean 5mAI: 0.041, range: 0.0 – 1.0, SE: 0.001). Three parous females came into estrus at different points in the study; the number of estrous females per month was low (range: 0 – 2) owing to a high proportion of lactating females in the study population.

Monthly party networks were dynamic over time (Figs 2.1 – 2.2, A.2 – A.3) and network density ranged from 0.14 (Jan) to 0.42 (Apr). Party networks for consecutive months were highly correlated (Fig. A.3), but correlation coefficients decayed as the time lag increased, indicating that party networks were locally stable within 2 – 3 month periods but were dynamic on a longer time scale. The 5m-network density ranged from 0.03 (Mar) to 0.06 (Jan) (Figs 2.2, A.4). There was no significant relationship between monthly party network density and monthly 5m-network density ($R^2 = 0.17$, $P = 0.270$; Fig. 2.2 inset). Variance-to-mean ratios of total edge weights per individual (i.e., weighted degree centrality) for party and 5m-networks were relatively low across months (party network: 3.52, 5m-network: 0.73; Fig. A.5), and while monthly party networks were significantly more aggregated than 5m-networks ($t_{8.3} = 4.38$, $P = 0.002$), degree distributions indicated that networks were not highly aggregated at either scale (Figs A.6 – A.7).

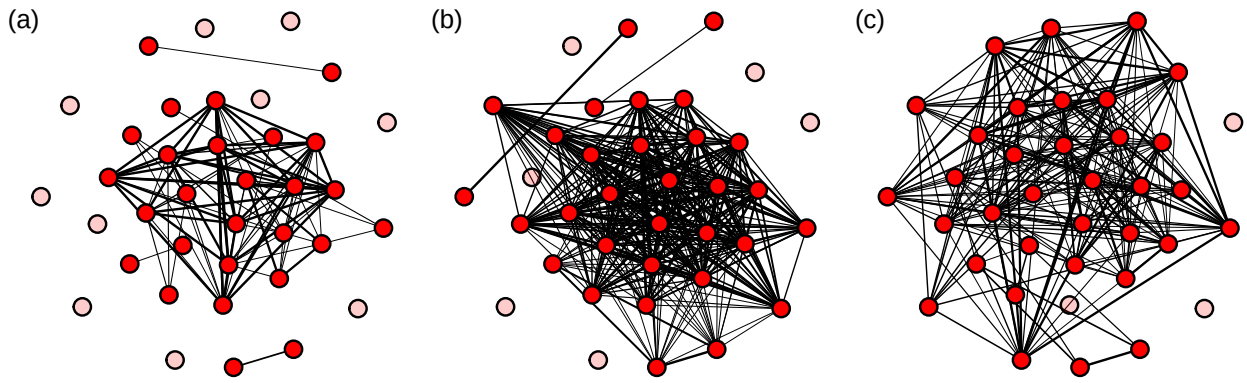

Figure 2.1: Monthly party association networks for a month with a) no estrous females (March), b) one estrous female (June), and c) two estrous females (August). Nodes (circles) represent individual chimpanzees ($n = 37$) and edges (lines) represent observed associations, where edge thickness corresponds to the pairwise party association indices (PAIs). All networks are displayed with identical layouts and only edges with PAIs $>$ 0.35 are shown. Dark red nodes have at least one edge above the PAI cutoff whereas light red nodes do not have any edges above the PAI cutoff. All nine monthly party association networks are shown in Fig. A.2.

Effects of social and ecological factors on pairwise associations

The number of estrous females in a given month significantly increased pairwise associations at the party-level, where for each additional estrous female, the odds of a pair associating were roughly twice as high (Table 2.1, Fig. 2.3). There was a significant interaction between the number of estrous females and age, such that adult-adult pairs experienced the largest increase in associations as the number of females in estrus increased. Similarly, of all the pairwise sex combinations, pairs that included one estrous female associated the most frequently. The odds of related pairs being in a party together were over 20 times greater than the odds for unrelated pairs, and chimpanzees were significantly more likely to associate with individuals of their own rank category. Family size difference negatively affected associ-

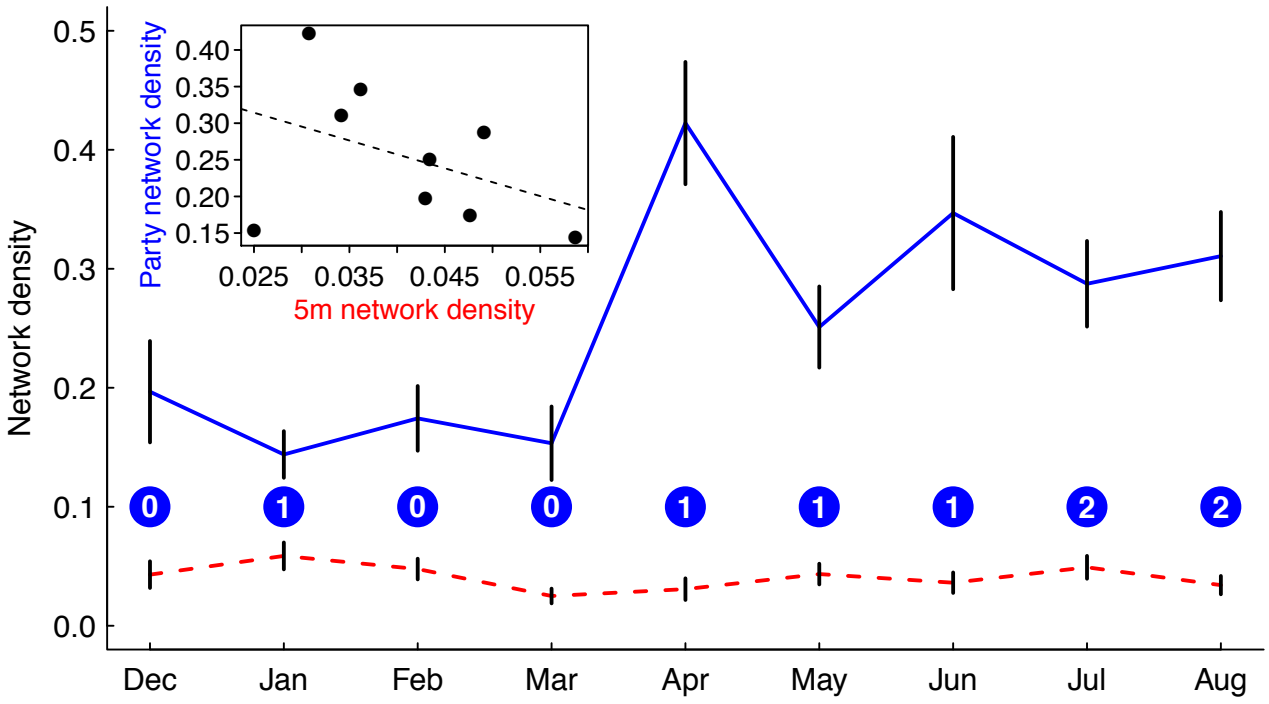

Figure 2.2: Density of monthly party networks (blue solid line) and 5m-networks (red dashed line) with standard error bars. The inset shows that there is no significant relationship between monthly party network density and monthly 5m-network density (Spearman Rank Test: $\rho =$ - 0.4, $P = 0.291$). Circled numbers show the number of estrous females in each month.

ation indices, indicating that individuals with large families (i.e., 3 – 4 members, Table A.4) tended to associate with other large families, and individuals without family units tended to associate with each other (Table 2.1).

The final model for pairwise party associations included random effects of chimpanzee ID and pair ID. Month was not included as a fixed or random effect, as the number of estrous females per month was a better predictor of monthly pairwise associations than month per se, based on the relative deviance information criterion, DIC (Δ DIC > 50). Rainfall lag and fruit availability parameters were removed because their exclusion increased model fit

Table 2.1: Effect of social factors on pairwise associations in party networks. The posterior mean, 95% credible interval, P-value based on MCMC sampling, and odds ratios (OR) are shown for fixed effect parameters. Bolded relationships are significant at P < 0.05. Sex/estrus and age categories are abbreviated as follows: age (adult: adult, AA; adult: juvenile, AJ; juvenile: juvenile, JJ), sex/estrus (pairwise combinations of male (M), female in estrus (Fe) and female not in estrus (F)).

Factor	**Posterior Mean**	**95% CI**	***P***	**OR**
Intercept	-3.58	-4.90, -2.22	**<0.001**	
Related	3.01	2.63, 3.39	**<0.001**	20.2
Sex (M:F)	0.73	-0.11, 1.57	0.087	2.07
Sex (M:M)	1.3	-0.38, 2.92	0.119	3.67
Sex (F:Fe)	1.76	1.24, 2.28	**<0.001**	5.83
Sex (M:Fe)	2.67	1.72, 3.65	**<0.001**	14.44
Difference in family size	-0.13	-0.20, -0.06	**<0.001**	0.88
Difference in rank	-1.04	-1.21, -0.86	**<0.001**	0.35
Age (AJ)	0.69	-0.23, 1.55	0.125	1.99
Age (JJ)	1.16	-0.59, 2.92	0.191	3.10
Number of estrous females	0.98	0.84, 1.12	**<0.001**	2.65
Number of estrous females:Age (AJ)	-0.22	-0.40, -0.02	**0.025**	2.14
Number of estrous females:Age (JJ)	-0.44	-0.72, -0.16	**0.003**	1.70

(rainfall: Δ DIC > 30, fruit: Δ DIC > 20, see Appendix Text A.1.4 for discussion of fruit availability and network structure). The final model had R^2 values that ranged from 0.32 – 0.58 for the amount of variation explained in each of the monthly networks, with the exception of August ($R^2 = 0.07$; Fig. A.8).

Results for 5mAIs were similar to the party-level results, although several variables in the 5m-model were significant in some but not all months (Table A.5). A major difference between these two levels of association was that pairs including an estrous female were often less likely to associate within 5m (as compared to pairs including an estrous female being more likely to associate at the party-level). As a second key difference, month was included as a fixed effect variable that interacted with every other fixed effect variable (age, sex/estrus,

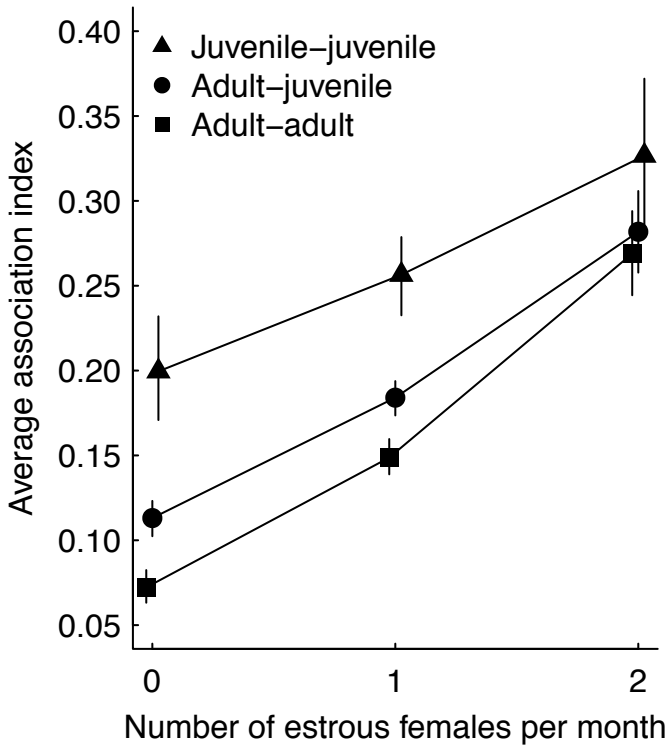

Figure 2.3: Estimated effect of estrous events on pairwise party associations. Model estimates of average association indices are shown for the three age-pair combinations with 95% credible intervals. The x-axis shows the number of females in estrus for a given month. Age combinations of adult-adult, adult-juvenile, and juvenile-juvenile pairs are represented by squares, circles, and triangles respectively. Figure estimates were calculated from the MCMC posterior distributions, while holding the presented parameters constant and allowing all other parameters to range across their possible values.

relatedness, family size difference, rank category difference), allowing the coefficients of these variables to vary for each monthly network (Table A.5). While more challenging to interpret, this final model fitted the data much better than the model including month as a random (and hence, additive) effect (Δ DIC > 100), or excluding month and including the number of estrous females to describe monthly change (Δ DIC > 350). This indicates that the number of estrous females was not as good of a predictor for 5m-associations as it was for party

	Party association networks, N=294						5m association networks, N=294					
	Degree		Eigenvector		Flow-betweenness		Degree		Eigenvector		Flow-betweenness	
	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*
Intercept	17.36	**<0.001**	0.17	0.269	33.41	0.058	1.35	0.096	0.13	0.324	35.52	0.109
Rank: M2	-1.43	0.108	-0.03	0.052	-1.41	0.212	-0.23	0.153	-0.04	0.085	-0.75	0.449
Rank: M3	-1.54	0.112	-0.02	0.078	-2.11	0.135	-0.65	**0.004**	-0.05	0.028	-0.15	0.496
Rank: F1	-1.19	0.150	-0.01	0.230	-3.51	0.023	-0.18	0.209	-0.02	0.271	-5.08	0.163
Rank: J1	-0.82	0.253	0.00	0.389	-3.74	0.019	-0.28	0.112	-0.02	0.221	-3.49	0.255
Rank: F2	-5.30	**<0.001**	-0.09	**<0.001**	-6.91	**<0.001**	-1.20	**<0.001**	-0.12	**<0.001**	-7.43	0.074
Rank: J2	-4.47	**<0.001**	-0.08	**<0.001**	-5.82	**0.002**	-1.13	**<0.001**	-0.13	**<0.001**	1.57	0.389
Estrus	0.85	0.324	0.01	0.404	0.24	0.443	0.30	0.204	0.05	0.102	3.72	0.272
Family size	0.73	**0.008**	0.01	**<0.001**	0.57	0.114	0.18	**0.001**	0.02	**0.001**	-0.50	0.356
R^2	0.618	**<0.001**	0.347	**<0.001**	0.195	**<0.001**	0.406	**<0.001**	0.251	**<0.001**	0.037	0.835

Table 2.2: Effect of social factors on party and 5m-association network centrality measures. Coefficients (β) and P-values are presented. Bolded values indicate significant relationships after Bonferroni correction. R^2 values are shown for each test. P-values for rank post-hoc significance tests are in Table A.6. Coefficients and P-values for month parameters are presented in Table A.7.

associations. The incorporation of month as a fixed effect precluded testing temporal variables (i.e., fruit availability, rainfall, and number of estrous females per month). Monthly R^2 values for the final 5m-association model ranged between 0.18 – 0.53 (mean: 0.34; Fig. A.8).

Predictors of individual centrality

Family size and dominance rank were the most important predictors for individual centrality at both the party and 5m-levels after controlling for the month of observation (Table 2.2; Figs 2.4, A.9). Adult females and juveniles with large families (i.e., 3-4 members) had significantly higher degree and eigenvector centrality; however, family size was not an important predictor for an individual's flow-betweenness centrality. This indicates that chimpanzees with large families had more edges and associated with other well-connected individuals, but were not more likely than random to connect two other individuals in the community.

Regarding rank, in both the party and 5m-association networks edge-ranging females and juveniles (F2 and J2) had significantly lower degree and eigenvector centrality than all other

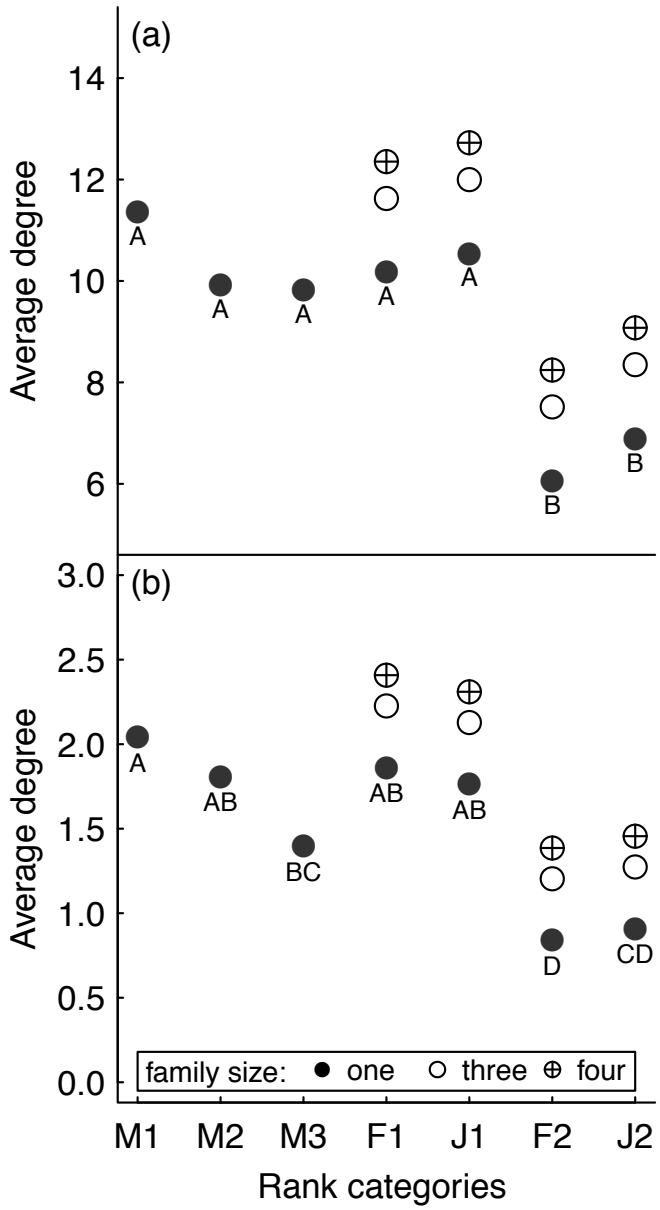

Figure 2.4: Estimated effects of rank and family size on average degree for (a) party and (b) 5m-networks. There was a significant positive relationship between an individual's family size and degree centrality. Black, white, and cross-symbol circles represent model estimates for individuals with family sizes of one, three, and four members respectively (by definition of family unit, adult male ranks are only presented with a family size of one). Letters on plots show which rank categories were significantly different (where overlap in letters between two rank categories indicates no significant difference after Bonferroni correction), after controlling for family size and estrous status.

ranks in both party and 5m-networks (with the exception that J2 did not have significantly lower degree centrality than low-ranking males in 5m-networks after Bonferroni correction; Figs 2.4, A.9; Table A.6). F2 and J2 also had significantly lower flow-betweenness centrality in party networks than high- and medium-ranking males. Altogether, these results indicate that edge-ranging adult females and juveniles were less connected to others and had fewer well-connected associates than all other ranks. They were also less likely than adult males to connect two random individuals in the party networks.

In 5m networks, high-ranking males (M1) had significantly higher degree centrality than low-ranking males (M3) (Figs 2.4, A.9; Table A.6). Additionally, there was a strong trend ($P < 0.05$) for M1 to have higher eigenvector centrality than M3 in 5m networks and higher flow-betweenness than core-ranging adult females and juveniles (F1, J1) in party networks; however, these differences were not significant after Bonferroni correction. Thus, while edge-ranging females and juveniles were nearly always the least central to the community, the relationship between high-ranking males and centrality was weaker and less consistent, with high-ranking males being significantly more central than other community members (e.g., M3, F2, J2) for some but not all centrality measures (Figs 2.4, A.9; Table A.6). Lastly, estrous status was never significantly related to centrality in party or 5m-networks (Table 2.2).

2.5 Discussion

Association patterns and insights for disease transmission

Our results demonstrate inter-individual and temporal variation in association patterns of wild chimpanzees, which should have profound effects on pathogen transmission dynamics. A main advantage of network analysis over more traditional connectivity measures, such as party size, is that network analysis explicitly quantifies how connectivity varies in relation to demographic and behavioral traits, and among individuals in a community. Degree dis-

tributions demonstrated that neither party nor 5m-networks were highly aggregated (i.e., most individuals had moderate centrality as opposed to a few superspreaders accounting for a majority of contacts); yet certain types of individuals had significantly higher association rates than others.

Adult females and juveniles with large families (i.e., 3 – 4 family members) were significantly more central than expected by chance in both party and 5m-networks, and individuals in core-ranging families were significantly more central than those in edge-ranging families. Thus, core-ranging adult females and juveniles with large families were the most central to the community. Additionally, chimpanzees associated more frequently with related individuals and individuals that had similar family sizes. Therefore, it seems that core-ranging chimpanzees with large families associated frequently with family members and also formed what Goodall (1986) referred to as nursing parties, where mothers and juveniles of different family units socialize together. Notably, there is evidence in West African chimpanzees (Taï Forest) that young juveniles maintain respiratory diseases in the community through play or close contact (Kuehl et al. 2008), a dynamic that has been demonstrated among human children for various childhood diseases (e.g., Fine and Clarkson 1982). Edge-ranging families were nearly always the least central to the community. In fact, the average degree centrality between a core-ranging adult female with a large family and an edge-ranging adult female without any juvenile offspring differed roughly by a factor of 2 in party networks and 2.5 in 5m-networks. Thus, individuals from edge-ranging families were the least likely to contribute to or be affected by pathogen transmission (although peripheral individuals could be exposed to pathogens from other communities or human settlements that overlap with forest edges).

Among core-ranging individuals, the average centrality of an adult female chimpanzee with three juveniles was roughly 2.5 degrees higher than that of an adult female with no juveniles. Previous wildlife network studies have demonstrated that even small differences in centrality can be linked to key differences in individual infection status. For example, a

study examining parasites in gidgee skinks (*Egernia stokesii*) determined that while network centrality was an effective predictor of parasite burden, the average difference in centrality between skinks with and without ticks was only ≈ 0.35 degrees (Godfrey et al. 2009). Thus, while we recognize that the magnitude of centrality metrics (which depend on network size and system-specific association definitions) should not be directly compared across systems, the significant increase we observed in chimpanzee centrality due to family size (even if modest in magnitude) could have a crucial impact on individual infection status.

While not as consistently central as core-ranging adult females and juveniles with large families, high-ranking males also had high centrality. Past work on the same study community showed high-ranking males tend to have increased levels of immunosuppressing testosterone (Muller and Wrangham 2004), and work in a nearby chimpanzee community (Ngogo) recently demonstrated that high-ranking males had both increased testosterone levels and greater helminth burdens (Muehlenbein and Watts 2010). Thus, in combination with the well-established immunosuppressive effects of sex hormones, their moderately central location in the network should make high-ranking males susceptible to contracting and transmitting a variety of pathogens. Taken altogether, we expect that core-ranging chimpanzees with large families, and to a lesser extent high-ranking males, should play an important role in pathogen transmission.

Contrary to our predictions, estrous females were not significantly more central than expected by chance in party or 5m-networks. This is surprising considering that among party networks, pairs including estrous females had higher levels of association and estrous females significantly increased association patterns across the community. Because a majority of adult females in our study community were nursing infants, the sample size for estrous females was limited ($n = 3$). Furthermore, one estrous female was frequently absent from the community and was presumed to be engaging in consortships, in which a mating pair travels away from the community (Goodall 1986). In future studies of centrality with larger

samples of estrous females, it may be necessary to develop networks that span shorter time frames (i.e., the length of maximal swelling, or roughly one week), as examining longer time steps includes times when the female does not have an estrous swelling and is potentially experiencing lower centrality.

While often overlooked in epidemiological analysis, temporal changes in behavioral interactions can affect outbreak timing (Altizer et al. 2006), as demonstrated by peaks in measles transmission in children during school sessions (Fine and Clarkson 1982) or by phocine distemper outbreaks coinciding with the haul-out behavior of seals (Swinton et al. 1998). Chimpanzee pairs were twice as likely to associate and party networks were denser when females were in estrus, suggesting that estrous events represent times of high vulnerability to infectious disease outbreaks. This result confirms findings from long-term field studies showing that chimpanzee party size increases with the number of estrous females (e.g., Wrangham 2000). Notably, there was no significant relationship between party and 5m-network density, and the number of estrous females did not significantly affect 5m-level associations. Thus, our network analyses suggest that the potential risk of outbreaks from pathogens that require very close contact for transmission might not increase with estrous events.

Implications for conservation and pathogen management

Epidemiological modeling studies in humans have shown that targeting central individuals for control efforts is significantly more effective in mitigating disease than applying control efforts randomly (Lloyd-Smith et al. 2005; Salathé et al. 2010). In a handful of cases, vaccination has been used to reduce the impact of emergent epidemics in endangered wildlife populations (Haydon et al. 2006; Woodford et al. 2002). Given the detrimental impacts of pathogens on great ape communities (e.g., Bermejo et al. 2006; Caillaud et al. 2006; Köndgen et al. 2008), some wildlife biologists have called for vaccinating great apes prophylactically

for high-risk pathogens (Ryan and Walsh 2011). To effectively plan control strategies and minimize human interference, network models can indicate the minimum number of well-connected individuals that should be vaccinated to reduce outbreak sizes (as per: Salathé et al. 2010). Importantly, using coarser connectivity metrics such as party size or group membership to parameterize infectious disease models would only capture a fraction of the contact heterogeneity observed in the networks described here. Our next steps include using Susceptible-Infected-Recovered (SIR) bond percolation models (Meyers 2007; Newman 2010) to simulate pathogen transmission on the observed monthly chimpanzee networks to assess the effectiveness of different intervention strategies in mitigating epidemics (such as targeting core-ranging individuals with large families for vaccination). This work is already underway with results from these simulations showing that moderately infectious pathogens (e.g., influenza) starting in core-ranging adult females and juveniles with large families are likely to generate significantly larger outbreaks than infections starting in other individuals (J. Rushmore, unpublished data).

Our findings are limited by examining a single chimpanzee community, and we recognize the need for similar analyses at additional field sites to provide a more comprehensive framework for designing disease management plans. Notably, the association data necessary for network analyses are likely available in long-term databases for many habituated wild ape communities. We encourage additional researchers to analyze such association data with a focus on potential pathogen transmission routes. In conclusion, our findings demonstrate temporal and inter-individual variation in association patterns for a wild chimpanzee community, and highlight how such behavioral variation could be incorporated into the development of disease management strategies for an endangered wildlife population.

2.6 Acknowledgements

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Chapter 3

NETWORK-BASED VACCINATION IMPROVES PROSPECTS FOR DISEASE CONTROL IN WILD CHIMPANZEES

J. Rushmore, D. Caillaud, R. Hall, R. M. Stumpf, L. A. Meyers, and S. Altizer. To be submitted to *Proceedings of the National Academy of Science*.

3.1 Abstract

Many endangered wildlife populations are vulnerable to infectious diseases for which vaccines exist; yet, pragmatic considerations often preclude large-scale vaccination efforts. These barriers can potentially be reduced by focusing on individuals with the highest contact rates. However, the question then becomes whether targeted vaccination is sufficient to achieve herd immunity. To evaluate the efficacy of targeted wildlife vaccinations, we simulate pathogen transmission and control on monthly association networks informed by behavioral data from a wild chimpanzee community (Kanyawara, Kibale National Park, Uganda). Despite considerable variation across monthly networks, we find that targeted vaccinations greatly reduce the required level of coverage as compared to a random (null) vaccination strategy. Specifically, our simulations indicate that using network data (degree centrality) to target the most connected individuals can reduce the number of chimpanzees requiring vaccination by up to 35% as compared to random vaccination. Because transmission heterogeneities may be attributed to biological differences among individuals (sex, age, dominance, and family size), we also evaluate the effectiveness of a trait-based vaccination strategy. The key benefit of this approach is that trait data are often easier to collect than data on individual positions in a social network. As compared to random vaccinations, our simulations suggest that the trait-based strategy can reduce the number of chimpanzees requiring vaccination by up to 18%, demonstrating that individual traits can serve as effective estimates of connectivity. Overall, our work indicates that parameterizing epidemiological models with fine-scale behavioral data can help optimize pathogen control efforts for endangered wildlife.

Key words: contact networks, disease management, epidemiological modeling, *Pan troglodytes*, pathogen dynamics, wildlife conservation

3.2 Introduction

Vaccines exist for many infectious diseases that threaten wildlife populations (Haydon et al. 2006; Ryan and Walsh 2011), yet immunization is rarely implemented as a conservation strategy. This is partly due to logistical difficulties in administering vaccines to large portions of wildlife populations. In particular, models based on homogeneous mixing typically indicate that a majority of individuals must be vaccinated to eliminate most pathogens (Anderson and May 1991). Further, because vaccination is economically costly and can carry its own risks (Wobeser 2007), high coverage levels can be infeasible or undesirable, particularly when dealing with endangered animals. For many wildlife species, individuals vary in contact rates (e.g., Lusseau 2003). Thus, to achieve herd immunity, control efforts focused on animals with the highest contact rates are expected to require considerably less coverage than random control (Meyers 2007).

Data on wildlife transmission and infection are challenging to collect, as darting or anesthetizing a sufficient number of individuals to assess pathogen prevalence can pose safety risks for humans and wildlife. Further, because epidemics can sweep through populations quickly, researchers often have inadequate time to obtain comprehensive health data (Leroy et al. 2004). On the other hand, behavioral association data, which can provide useful estimates for transmission pathways (Salathé et al. 2010), is readily collected through behavioral observation or tracking devices (Hamede et al. 2009). While rarely applied to wildlife systems, network epidemiology (in which nodes represent individuals and edges represent interactions allowing for pathogen transmission) is a powerful tool that can be applied to association data to mathematically represent heterogeneity among transmission pathways (Craft and Caillaud 2011). We present here the first application of network-based pathogen control strategies to a wild ape species; our results indicate that network-based vaccinations require markedly less coverage than random vaccinations to curtail outbreaks in a wild chimpanzee

community.

Great apes have experienced considerable declines from infectious diseases such as Ebola and respiratory viruses (Bermejo et al. 2006; Köndgen et al. 2008). Apes also demonstrate substantial heterogeneity in individual contact rates that can induce tremendous individual variation in the risk of acquiring or spreading infections (Caillaud et al. 2006). In particular, chimpanzees have highly structured, fission-fusion societies, where individuals within communities break off into smaller parties of variable size and composition over days to months (Goodall 1986). Previous work on the same population examined here showed high variability in contact rates over time and across individuals (Rushmore et al. In review), with individuals with large families (i.e., adult females and juveniles) that range in the core area of the community territory being consistently more central to the community than other chimpanzees. Thus, for populations where network data are not available, targeting individuals with easily identifiable social traits (as a proxy for network connectedness) may reduce coverage levels as compared to random vaccination.

We investigated how contact heterogeneity within a chimpanzee community affects dynamics of pathogen transmission and control using network epidemiology and empirically-derived contact networks. Specifically, we simulated pathogen spread on a series of monthly contact networks to assess how final outbreak sizes were affected by i) the network position of the index case (first individual to be infected), ii) the timing of the initial case (month), and iii) pathogen infectiousness. Our simulations used estimated values of the basic reproductive number (R_0) reported in the human literature for a range of mild to highly infectious pathogens (Anderson and May 1991). We then identified key traits of individuals likely to initiate large outbreaks (similar to high-risk groups identified for human diseases such as HIV), and we compared the effectiveness of a random (null) vaccination strategy versus two network-based vaccination strategies: i) vaccinations based on network centrality data and ii) vaccinations targeting individuals with high-risk social traits. We predicted that final

outbreak size would increase with index case centrality and that infections starting in core-ranging adult females and juveniles with large families would lead to the largest outbreaks. We also expected the centrality-based targeted vaccinations to require the least coverage to mitigate outbreaks; however, the trait-based vaccinations might be the most practical to implement in the field.

3.3 Results

Effects of network heterogeneity on outbreak size

The network position of the index case, the month of the initial case, and pathogen infectiousness strongly affected mean outbreak size for the chimpanzee community in our simulations. We estimated outbreak size (the cumulative number of individuals infected during an outbreak) for every combination of index case ($n = 37$), month ($n = 9$), and pathogen infectiousness ($n = 4$) by stochastically simulating pathogen transmission on observed contact networks using bond percolation (Methods, Appendix Text B.1.1). Our models used basic reproductive numbers averaged across monthly networks ($\overline{R_0}$) for a range of values of pathogen infectiousness (from mildly contagious: $\overline{R_0} = 0.7$, to highly contagious: $\overline{R_0} = 10$; Methods, Appendix Text B.1.1).

Network density (mean edge weight) varied across months by a factor of 2.4 (Appendix B: Fig. B.1), which greatly influenced mean outbreak size (e.g., outbreak sizes varied across months by a factor of 3.0 when $\overline{R_0} = 0.7$ and by a factor of 1.8 when $\overline{R_0} = 10$). Outbreak size generally increased with monthly network density, with stronger relationships for low and moderate $\overline{R_0}$ values (Fig. B.1), as higher $\overline{R_0}$ values had large outbreak sizes for most months. Additionally, time series chain-binomial models (which were run for a subset of network months and index cases: Methods) revealed that outbreaks parameterized with estimates for moderate or highly infectious pathogens (e.g., influenza or measles) lasted less

than a month (Fig. B.2). This finding suggests that the one-month static networks (which were significantly correlated with the corresponding two-week static networks: Rushmore et al. In review) used in our simulations should capture host interactions at a temporal scale that reflects realistic pathogen transmission.

Within a given monthly network, mean outbreak size increased with node centrality of the index case. This was the case for three weighted centrality measures (degree, eigenvector, and betweenness: Methods) across all $\overline{R_0}$ values, with higher correlations for lower $\overline{R_0}$ values (e.g., degree centrality: for $\overline{R_0} = 0.7$, $R^2 = 0.97$; for $\overline{R_0} = 10.0$, $R^2 = 0.52$; Figs. 3.1, B.3). Notably, the extent of contact variation within and across months allowed mildly infectious pathogens ($\overline{R_0} = 0.7$) to affect up to 30% of the community, provided that the infection started with a highly central index case, whereas outbreaks of highly infectious pathogens ($\overline{R_0} = 10$) could be avoided if the infection started in a less central index case (Fig. 3.1). Additionally, the mean outbreak size linked to an index case varied across months, as a less central individual in one month could be a moderately central individual in another month (Fig. 3.1). The dynamic nature of the contact structure may thus appear as an obstacle to efficient network-based vaccinations. However, for our study system, degree centrality was the best predictor of mean outbreak size (across all $\overline{R_0}$ values: Fig. B.4), and average degree centrality of the index case was strongly correlated with outbreak size. Thus, vaccination based on average degree should be robust to monthly network variation.

Parameterizing vaccination strategies

As compared to a random (null) vaccination strategy, we investigated the efficacy of two network-based vaccination approaches: i) targeting individuals in order from high to low average degree centrality, and ii) targeting individuals based on social traits that predict high centrality. Rushmore et al. (In review) showed that the most important predictors for individual centrality in our study community were family size and range location (core

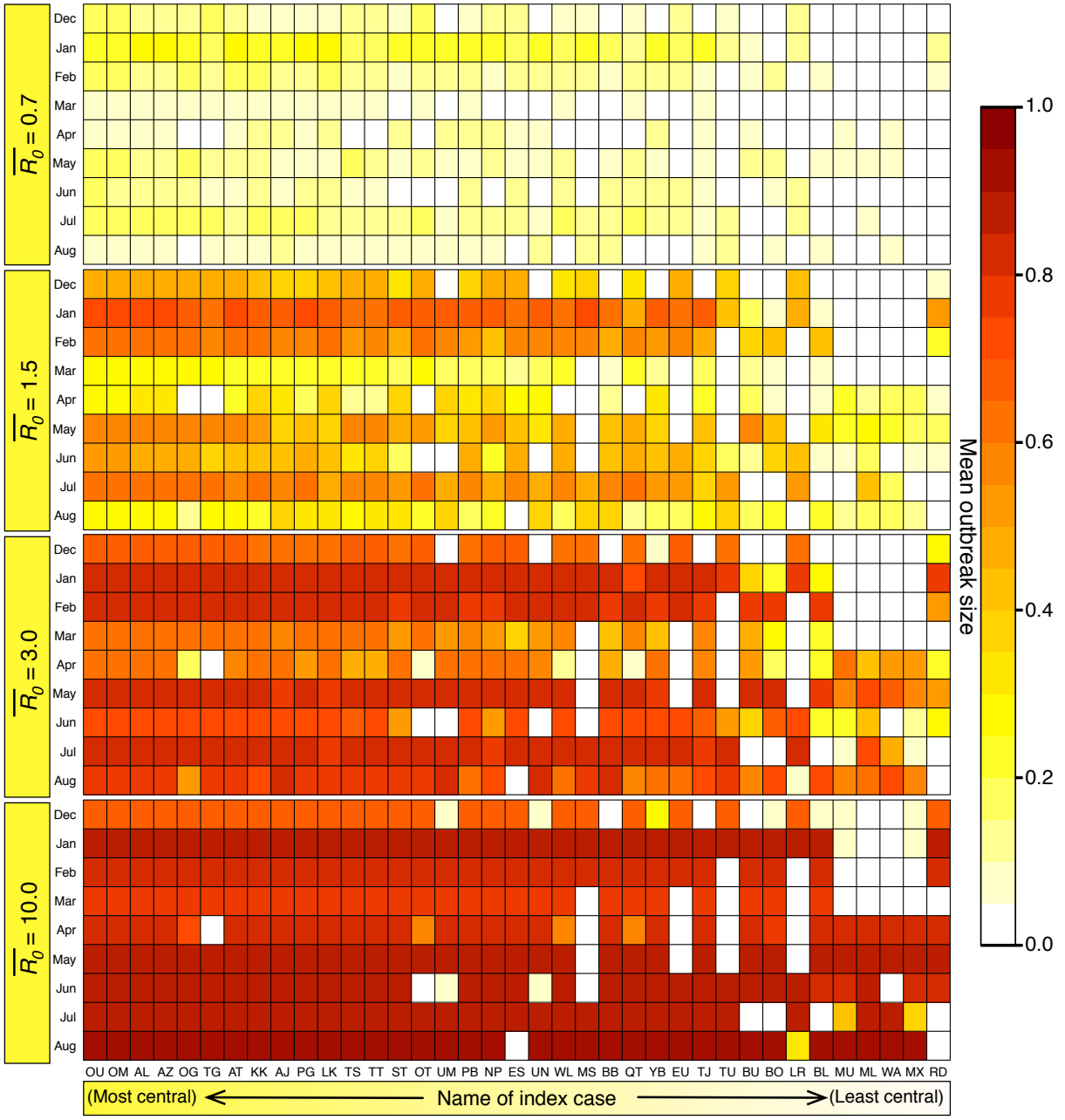

Figure 3.1: Mean outbreak size as a function of index case, month of initial case, and pathogen infectiousness ($\overline{R_0}$). The color of each cell shows the average proportion of the chimpanzee community ($n = 37$) that was infected across the 1000 replicates per unique combination of parameters. The x-axis shows the identities of the index cases, ordered from highest to lowest mean degree centrality (i.e., averaged across months).

versus edge) for adult female and juvenile groups, and dominance rank for males. Thus, we placed individuals in trait-based groups (Methods, Fig. 3.2, Table B.1) and verified that our classification scheme predicted outbreak sizes using permutation-based regression tests (Methods, Appendix Text B.1.1). Results showed that for adult females and juveniles, infections starting in core-ranging individuals from large families (hereafter, CR-L) led to significantly larger outbreaks across all $\overline{R_0}$ values than infections starting in core-ranging individuals with small families (CR-S) or edge-ranging (ER) individuals (Fig. 3.3). Infections starting in ER individuals led to significantly smaller outbreaks than for any other group, making ER individuals poor targets for vaccination (Fig. 3.3). For low $\overline{R_0}$ values, high rank (HM) adult male index cases tended to cause larger outbreaks than mid- (MM) or low-ranking (LM) adult males but this pattern was not consistent across $\overline{R_0}$ values, and differences were never significant. Thus, we collapsed HM, MM, and LM into a single adult male group (M), and found that infections originating in CR-L individuals, but not CR-S individuals, generally caused significantly larger outbreaks than infections starting in adult males (Fig. 3.3).

Given these findings, we parameterized the trait-based vaccination strategy to preferentially vaccinate in the following order: CR-L, M, CR-S, ER. Thus, for each simulation, individuals were first immunized randomly within the CR-L group. Once all individuals in this group were vaccinated, individuals in the M group were randomly immunized, and so on until the predetermined level of coverage was reached. To ensure that collapsing the adult male categories did not influence results, we also simulated vaccinations with the following order: CR-L, HM, MM, LM, CR-S, ER. The main text and figures present results for a single male category; however, results for both scenarios are shown in Table 3.1.

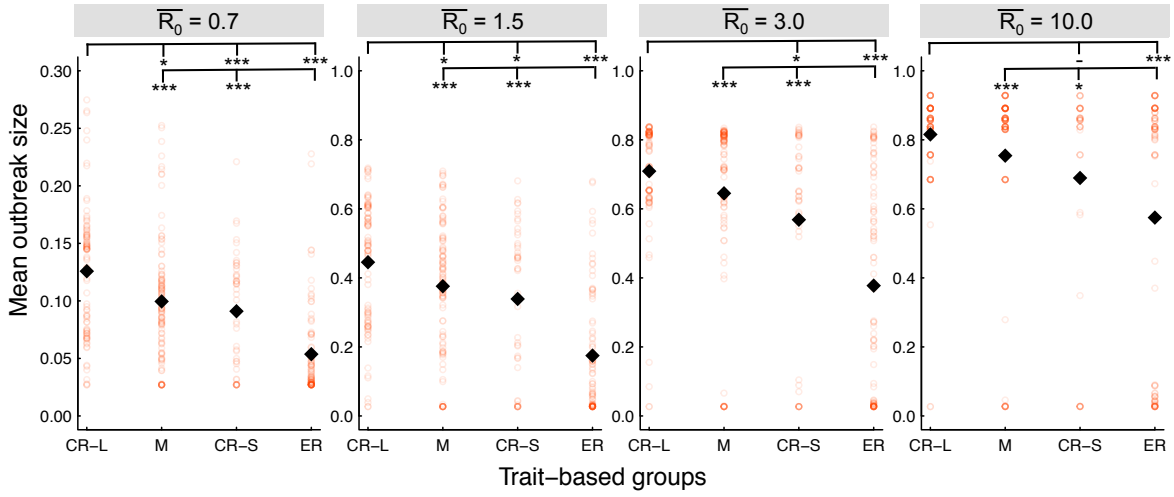

Figure 3.2: Mean outbreak size for index case trait-based groups across different values of pathogen infectiousness ($\overline{R_0}$). Mean outbreak size is shown as a proportion of the whole community, and trait-based groups are abbreviated as follows: CR-L, core-ranging individuals with large families; M, adult males; CR-S, core-ranging individuals with small families; ER, edge-ranging individuals. Red circles show outbreak size averaged across 1000 simulations per unique combination of monthly network and index case for a given $\overline{R_0}$. Black diamonds mark the mean outbreak size averaged across each trait-based group. Note the different y-axis scale for the first panel ($\overline{R_0} = 0.7$). Significant relationships are indicated: (–) $P < 0.05$, (*) $P < 0.01$, (**) $P < 0.001$, (***) $P < 0.0001$.

Evaluation of vaccination strategies

We evaluated vaccination strategies in two ways: i) to assess coverage needed to protect against the central outbreak tendency (hereafter, the Minimum Coverage Threshold approach), we determined the coverage required to constrain the mean outbreak size to less than 10% of the community, ii) to assess coverage needed to protect against rare outbreak events (hereafter, the Conservative Coverage Threshold approach), we determined the coverage required to reduce at least 95% of the simulated outbreaks to less than 10% of the community. Constraining outbreaks to 30% of the community (instead of 10%) showed qualitatively similar results for both approaches (Table B.2). Results based on the Minimum

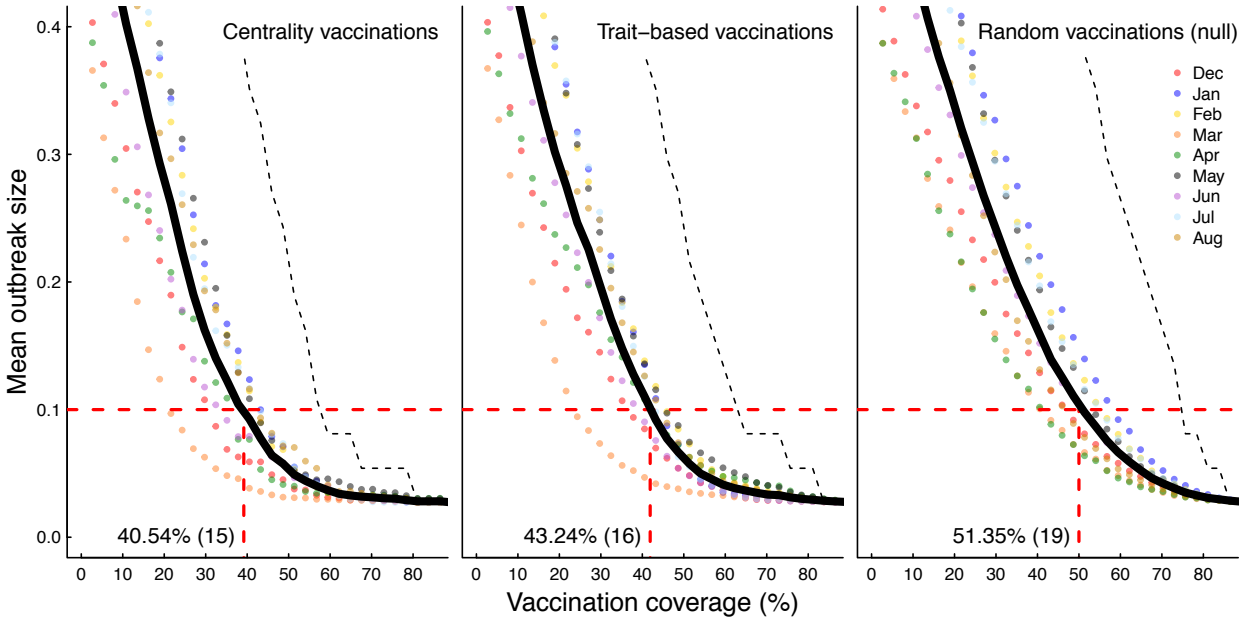

Figure 3.3: Evaluation of vaccination strategies by Minimum Coverage Threshold. Mean outbreak sizes (as proportions of the community) are shown for varying levels of vaccination coverage (as percentages of the community) when $\overline{R_0} = 3.0$. Colored dots show mean outbreak size by month, and thick black lines show mean outbreak size averaged across months. Red dotted lines indicate the Minimum Coverage Threshold (with the number of chimpanzees in parentheses) required to curb outbreaks to $< 10\%$ of the community. The dotted black lines show upper 95% confidence intervals, which are equivalent to the Conservative Coverage Thresholds depicted in Fig. 3.4. For all coverage levels and vaccination strategies, the lower 5% of simulations (not shown) had a mean outbreak size of 2.7%, indicating that only the index case was infected.

Coverage Threshold showed that pathogens with intermediate $\overline{R_0}$ values required randomly vaccinating between 30-50% of the community, and highly contagious pathogens required randomly vaccinating roughly 65% of the community (Table 3.1). By the Conservative Coverage Threshold, pathogens with intermediate $\overline{R_0}$ values required randomly vaccinating up to 75% of the community, with roughly 85% coverage required for a highly contagious pathogen (Table 3.1).

Across all $\overline{R_0}$ values, network-based strategies consistently required less coverage than

A. Minimum Coverage Threshold:				
Vaccination strategy	$\overline{R_0}$= 0.7	$\overline{R_0}$= 1.5	$\overline{R_0}$= 3.0	$\overline{R_0}$= 10.0
Centrality-based	0% (0)	24.32% (9)	40.54% (15)	56.76% (21)
Trait-based	0% (0)	29.72% (11)	43.24% (16)*	59.46% (22)
Random	0% (0)	35.13% (13)	51.35% (19)	64.86% (24)
B. Conservative Coverage Threshold:				
Vaccination strategy	$\overline{R_0}$= 0.7	$\overline{R_0}$= 1.5	$\overline{R_0}$= 3.0	$\overline{R_0}$= 10.0
Centrality-based	29.72% (11)	51.35% (19)	59.46% (22)	81.08% (30)
Trait-based	37.84% (14)*	54.05% (20)*	64.86% (24)	81.08% (30)**
Random	45.95% (17)	64.86% (24)	75.68% (28)	86.49% (32)

Table 3.1: Comparison of coverage thresholds across vaccination strategies and pathogen infectiousness. For each vaccination strategy, the coverage threshold is provided as a percentage of the community, with the number of individuals vaccinated in parentheses, for A) the mean outbreak size to affect $< 10\%$ of the community (Minimum Coverage Threshold), and B) an outbreak to affect $< 10\%$ of the community in at least 95% of the simulations (Conservative Coverage Threshold). The table shows results for trait-based simulations using a single adult male category (M). Results were identical for simulations using this category M or three adult male categories (HM, MM, LM; see Results), except for a few instances, denoted by superscripts (*) and (**) in which simulations using HM, MM, and LM categories required vaccinating one less or one more individual, respectively.

random vaccinations to achieve the same level of protection; although, network-based strategies offered the greatest advantage for pathogens with low to moderate infectiousness (Figs 3.3-3.4, B.6; Tables 3.1, B.2). While centrality- and trait-based strategies occasionally performed equivalently, centrality-based vaccinations typically required less coverage (Figs 3.4, B.6; Tables 3.1, B.2). The Conservative Coverage Threshold showed that as compared to random vaccinations, the number of individuals requiring vaccination was reduced by up to 17.65% with the trait-based strategy and by up to 35.29% with the centrality-based approach. Lastly, because each vaccination strategy uniquely changed the underlying network for a given month by removing immunized nodes and adjacent edges, responses to the three vaccination strategies differed across months. For example, when $\overline{R_0} = 3.0$, April required twice as much

coverage as March with network-based strategies, whereas the two months required equal coverage with random vaccinations (Fig. 3.3). Nonetheless, network-based vaccinations were considerably more effective than random control when averaged across months.

3.4 Discussion

Our study provides support for the effects of temporal contact heterogeneity on pathogen transmission dynamics and shows that these variables play a crucial role in predicting outbreak probability and designing vaccination programs for endangered wildlife. Outbreaks were largest when the index case had high degree centrality, and these individuals were generally core-ranging females or juveniles with a large family. Compared to random control, simulated vaccinations that preferentially targeted individuals based on high-risk traits or degree centrality reduced the number of chimpanzees requiring vaccination by up to 18% and 35%, respectively. Thus, our simulations show that targeting individuals with high contact rates effectively reduces the level of vaccination coverage required to achieve herd immunity and could help make wildlife vaccination a more tractable pathogen control tool.

Models assuming homogeneous mixing show that outbreak size is larger for high R_0 values than for R_0 values close to one (Anderson and May 1991); however, our analysis showed this was not always the case when accounting for heterogeneous contact rates. We observed that outbreaks of mildly infectious pathogens could affect up to 30% of the community if introduced via highly connected individuals during a well-connected month, whereas an extremely infectious pathogen was unlikely to spread to anyone when starting in a peripheral index case during a sparsely connected month. These findings suggest that contact structure can play a fundamental role in pathogen emergence and evolution. For example, a mildly contagious pathogen could become more infectious through selection occurring during the early stages of an outbreak (Antia et al. 2003). Although a growing number of studies have

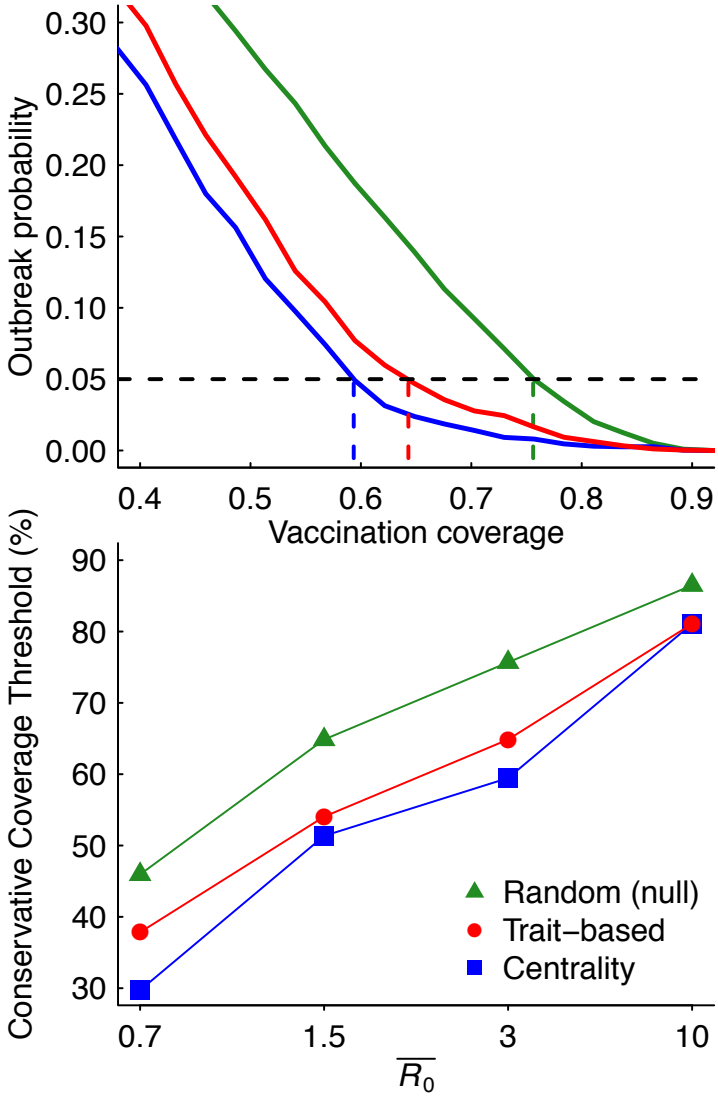

Figure 3.4: Evaluation of vaccination strategies by the Conservative Coverage Threshold. The top panel shows the outbreak probability (the proportion of simulations resulting in an outbreak greater than 10% of the community) for centrality-based vaccinations (blue), trait-based vaccinations (red), and random vaccinations (green) at varying levels of coverage (shown as a proportion of the community) when $\overline{R_0} = 3.0$. The black dotted line marks the Conservative Coverage Threshold, at which no more than 5% of the simulations result in outbreaks. The bottom panel shows this Conservative Coverage Threshold for each vaccination strategy and $\overline{R_0}$ combination.

used temporal contact variation to predict disease outbreaks for human populations (e.g., Stoddard et al. 2013), our study is one of the first to demonstrate these dynamics for an endangered wildlife species.

As compared to random vaccination, our analysis pointed to improved control strategies that could be implemented for communities both with and without network data. Given the extreme speed with which many pathogens spread through chimpanzee populations (both in our temporal simulations and in observed outbreaks: Hanamura et al. 2008; Williams et al. 2008), prophylactic vaccination is likely a more effective intervention option than treating sick individuals in the midst of an epidemic. Due to herd immunity, our results predict that even the least effective (random) strategy would not require vaccinating the entire community to prevent an outbreak. Of the random and network-based strategies we tested, the most effective method was prophylactically vaccinating individuals based on degree centrality; however, this strategy is only feasible for populations with readily available network data. For populations lacking such data, trait-based immunizations could offer a more practical approach, where wildlife managers could target individuals for vaccination based on traits known to be associated with high contact rates for a given species or population. In this small study community of 37 chimpanzees, the impacts of trait-based vaccinations on the total number of animals to be treated were somewhat modest. However, we expect that trait-based immunizations could substantially decrease the number of animals requiring vaccination for larger communities (such as the Ngogo chimpanzee community, $n \approx 150$, in Kibale Forest, Uganda: Mitani et al. 2010) or for large populations of other wildlife species. Moreover, as many vaccines are administered to wildlife via hypodermic dart, there is always some expense and risk associated with immunizing endangered animals. For example, the darted animals may experience wounds, falls, or adverse effects from the vaccine, and the veterinarian performing the darting could experience counterattacks or loss of trust from habituated animals. Thus, even moderate coverage reductions offer an extremely valuable

conservation advantage.

We recognize there are limitations of our study. In particular, further work is needed to determine if the traits associated with high network centrality here also apply to other habituated chimpanzee communities. Second, our simulations revealed that correlations between outbreak size and index case centrality were highest when pathogen infectiousness was low or moderate, indicating that the benefits of network-based immunizations over random vaccination could be minimal for highly contagious pathogens. Third, we were unable to identify temporal drivers for pathogen transmission in the proximity networks presented in the Results (but see Methods and Appendix B for evidence of a positive relationship between the number of estrous females and outbreak size in party networks). Nevertheless, our targeted vaccinations showed marked improvements over random control when averaged across months. Lastly, our models assumed that vaccinated individuals received full protection and that infected individuals had equal-length infectious periods. Future work aimed at relaxing these assumptions could help clarify the role that individual immunity plays in pathogen transmission dynamics and could further improve disease control efforts.

Our finding that network-based vaccinations require less coverage than random vaccinations should apply broadly to other social wildlife species. New technologies, such as proximity-logging collars, have made collecting fine-scale association data more feasible than ever, even for elusive or nocturnal wildlife (Hamede et al. 2009). Thus, our methods for developing and assessing network-based control could readily be adapted to other host systems. Further, because index case centrality was highly associated with outbreak size, our results indicate that contact rates generate useful predictions of which individuals to vaccinate, even in lieu of mechanistic modeling. Overall, we argue that incorporating temporal contact variation into epidemiological models can help optimize disease control efforts across a range of host systems, including many social wildlife species.

3.5 Methods

Field data collection

Over a nine-month period (Dec 2009 - Aug 2010), we collected behavioral contact data on the habituated, wild Kanyawara chimpanzee community ($n = 48$) in Kibale National Park, Uganda. Further information on the study site and community is provided in Appendix Text B.1.2. Each morning, we randomly selected a focal chimpanzee from a party (i.e., individuals within a 50m radius). At 15-minute intervals, we scanned the focal animal's party to record party member identities and pairs of individuals that were within 5m of each other. Our total sample size was 37 individuals (adults: 12 males and 12 females; juveniles: 7 males and 6 females), excluding dependent offspring (< 4 years old). On average, we followed each chimpanzee as a focal subject for 27.79 ($\pm$3.6) hours, comprising a total of 1,028 focal observation hours and 4,114 fifteen-minute scans. Our analysis included 306,212 pairwise party associations and 14,673 pairwise 5m-associations. See Rushmore et al. (In review) for full data collection details.

Quantifying contact networks

We created monthly contact networks at two spatial scales (proximity networks and party networks) across nine months, in which nodes represented chimpanzees. Proximity network edges were weighted by 5m association indices, which were based on the probability that a pair would be both within the same party and within 5m of each other in a given month (Appendix Text B.1.1). Hence, proximity networks were a proxy for transmission routes of pathogens spreading by direct contact or respiratory droplets. Alternatively, party network edges were weighted by party association indices, which were based on the frequency of monthly co-occurrence in the same party for a pair of chimpanzees (Appendix Text B.1.1). Thus, party networks were a proxy for pathogen transmission not requiring close contact

(via aerosol transmission or fomites). Because many pathogens require close contact for transmission, results in the main text are intended to pertain to proximity networks unless otherwise stated; party network results are in the supporting information (Figs. B.1, B.4 – B.6). Results were consistent across network scales, with the exception of a positive relationship between mean outbreak size and the number of estrous females for party networks (Fig. B.5), but not for proximity networks (Fig. B.3).

Individual trait data

Adult male chimpanzees tend to follow a linear hierarchy, which is not the case for eastern chimpanzee adult females (Goodall 1986). In the Kanywara community however, adult females that occupy the territory core tend to be higher ranking than those occupying the territory edges (Kahlenberg et al. 2008). Adult females and their juveniles typically travel in family units, but not females without juveniles or adult males (Goodall 1986). We considered large families to be a mother with two or more juveniles (Table B.1). Additional details are described in Rushmore et al. (In review).

Calculating centrality measures

For each individual in each month, we calculated the following weighted centrality measures: degree (the sum of a node's edge weights: Newman 2010), eigenvector centrality (a metric based on a node's connectedness and the connectedness of the nodes' associates: Newman 2010), and betweenness (the proportion of times an individual lies along the shortest path between pairs in the network: Freeman et al. 1991). To test for relationships between mean outbreak size and each of the index case centrality metrics, we used linear models that controlled for month.

Simulating pathogen transmission on observed networks

We simulated pathogen transmission on observed contact networks using bond percolation, a computationally tractable approach to estimate the final outbreak size of a stochastic, network-based Susceptible-Infected-Recovered (SIR) model (Meyers 2007; Newman 2010). In a bond percolation model, the probability of an infectious disease being transmitted (T) along an edge connecting nodes i and j, given that one of the nodes is infected, is related to the contact rate between individuals (c_{ij}), the pathogen transmission rate (β), and the infectious period (τ) as follows (Newman 2010):

$$T_{ij} = 1 - e^{-c_{ij}\beta\tau} \tag{3.1}$$

In our case of weighted networks, we parameterized c_{ij} using pairwise association indices from monthly networks. We assumed that for a given pathogen, the transmission rates and infectious periods were the same across all transmission events and individuals.

In each simulation, the bond percolation method simplified the observed network by removing edges (with probability 1-T) that would not lead to transmission events. The remaining graph represented possible transmission routes, where nodes connected to the index case represented individuals that became infected during the simulation. Therefore, the size of the component (i.e., connected network) with the index case was the final outbreak size. Unlike methods that reproduce temporal outbreak dynamics, such as chain-binomial models (Bailey 1957), bond percolation does not track temporal changes in individual infection status, and hence substantially reduces computational time relative to the chain binomial method. To check the validity of our models, we simulated time series pathogen transmission for a subset of parameter combinations using the chain-binomial method, which yielded identical results to our percolation-based simulations (Fig. B.2).

The basic reproductive number (pathogen infectiousness) depends on the probability of

transmission (T_{ij}) and network connectivity. In the specific case of our study population, we defined the basic reproductive number as the mean number of secondary infections that arise from a randomly infected index case, averaged across all nine monthly networks:

$$\overline{R_0} = \frac{1}{9}\sum_{m}\frac{1}{37}\sum_{i}\sum_{i\neq j}T_{ij} \tag{3.2}$$

where m represents each of the nine monthly networks at a given spatial scale (i.e., proximity versus party networks) and i and s are the indices of the 37 individuals (Appendix Text B.1.1). Averaging R_0 across monthly networks allowed us to measure the effect of network structure (month) on outbreak size, for a given level of pathogen infectiousness. We calculated pathogen transmission rate (β) so that the resulting $\overline{R_0}$ matched estimates for infectious pathogens reported in the human literature that showed potential for infecting wild apes (Appendix Text B.1.1). Specifically, we used values of $\overline{R_0} = 0.7$ (representing mildly contagious pathogens in which the average index case does not consistently infect at least one other individual), $\overline{R_0} = 1.5$ (moderately infectious pathogens, such as Ebola or influenza: Chowell et al. 2006, 2004), $\overline{R_0} = 3.0$ (moderately infectious pathogens, such as influenza: Mills et al. 2004), and $\overline{R_0} = 10$ (highly infectious pathogens, such as measles: Anderson and May 1991). Notably, these referenced R_0 values were calculated for a small number of human populations and should be extrapolated to other human and great ape populations with caution. Nevertheless, while the $\overline{R_0}$ for these diseases may vary slightly in our study population, their respective ranks should be consistent. Further information regarding our definition of $\overline{R_0}$, which differs slightly from another definition often used in network epidemiology, is in Appendix Text B.1.1.

To examine the effect of index case centrality, month of initial case, and pathogen infectiousness ($\overline{R_0} = 0.7$, 1.5, 3.0, 10.0) on outbreak size, we ran 1000 simulations per unique combination of these three parameters at two spatial scales (i.e., proximity networks and

party networks), resulting in 2,664,000 simulations. All simulations and subsequent analyses were run in R v. 2.15 (R Core Development Team 2010); code is available from J. Rushmore upon request.

Parameterizing trait-based vaccination strategies

To determine which individuals were associated with high outbreak sizes, we used permutation-based regressions with 30,000 permutations per test (Appendix Text B.1.1). While controlling for month and $\overline{R_0}$, we examined relationships between mean outbreak size and the trait-based group of the index case (e.g., based on the individual's rank and family size).

Simulating vaccination strategies

Using bond percolation as described above, we simulated vaccination strategies on observed monthly networks with the assumption that vaccination conferred full protection to treated individuals. For each strategy, we ran 5,000 simulations per unique combination of month, $\overline{R_0}$, and coverage level (which varied sequentially from 1 to 37 individuals).

3.6 Acknowledgements

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Chapter 4

SCREENING GREAT APES FOR PUTATIVE SEXUALLY TRANSMITTED DISEASES: EVIDENCE OF TRICHOMONADIDAE INFECTIONS IN WILD CHIMPANZEES

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4.1 Abstract

Sexually transmitted diseases (STDs) can persist endemically and are known to cause sterility and increased infant mortality in humans. STDs could have similar impacts in wildlife populations, yet studies to date are generally limited to cases where outward signs are apparent. African apes (i.e., chimpanzees, bonobos and to a lesser extent gorillas) show promiscuous mating behavior that could offer opportunities for STD transmission, yet little is known about the prevalence and impact of STDs for this endangered primate group. The goal of our study was to test African great apes for putative STDs that either commonly affect humans or were previously detected in captive apes. We screened biological samples from a total of 172 wild and orphaned eastern chimpanzees and gorillas for four classes of pathogens: trichomonads, *Chlamydia* spp., *Treponema pallidum* (syphilis and yaws), and papillomaviruses. All samples were negative for *Chlamydia*, *Treponema pallidum*, and papillomaviruses; however, a high percentage of wild chimpanzee urine and fecal samples showed evidence of trichomonads. Generalized linear models revealed that females were more likely than males to have positive urine, but not fecal, samples. Moreover, positive urine samples were more frequent during dry months whereas positive fecal samples were more common during wet months. Sequence analysis of positive urine samples and previously described genetically similar trichomonads revealed three trichomonad groups within the genus *Tetrarichomonas*, with newly generated sequences from our study occupying two groups. Evidence from other studies suggests that one sequence group is likely transmitted via fecal-oral routes, but the transmission of other sequences remains unclear. We encourage additional researchers to investigate great ape STDs, as this work could offer insights for the management of endangered great apes and for our understanding human STD origins.

Key words: *Chlamydia* spp., *Gorilla beringei*, *Pan troglodytes*, papillomavirus, *Tetratrichomonas* spp., *Treponema pallidum*, venereal disease, wildlife conservation

4.2 Introduction

While often overlooked in non-human hosts, sexually transmitted diseases (STDs) are widespread throughout the animal kingdom and can impact host reproduction and evolution (Lockhart et al. 1996; Smith and Dobson 1992). Knowledge of non-human STDs is largely focused on animals of economic value (e.g., pets or food animals: Cameron 1947; Carmichael and Kenney 1968), whereas relatively little is known about STDs in wildlife populations. Mating provides a key transfer route for STDs, and species with promiscuous mating systems are expected to harbor more STDs than monogamous species (Lockhart et al. 1996; Loehle 1995). African great apes (chimpanzees, *Pan troglodytes*; bonobos, *Pan paniscus*; and to lesser extent gorillas, *Gorilla beringei*) exhibit extreme promiscuity (Campbell et al. 2011), with estrous female chimpanzees mating up to 50 times in one day with several different males (Goodall 1986). In addition, the great apes are the closest living relatives to humans, a host species known to harbor a high diversity of STDs, including viruses, bacteria, protozoa and lice (Holmes et al. 2008).

STDs often show few outward signs (Holmes et al. 2008), which could result from selection favoring reduced virulence, as apparent or virulent afflictions could lower transmission by preventing infected hosts from pursuing or attracting mates (Knell 2004). Despite low impacts on host survival, many human and some animal STDs are known to cause persistent infections associated with sterility or infant mortality, suggesting a high cost to fecundity of infected hosts (Lockhart et al. 1996). Further, owing to their mode of transmission, models predict that STDs can persist in small, declining host populations (de Castro and Bolker 2005; Lockhart et al. 1996; Smith and Dobson 1992), and could pose unusually high risks for threatened populations. Given the endangered status of great apes (IUCN 2012) and the known effects of STDs on human sterility (Holmes et al. 2008), studies that examine the prevalence and impacts of STDs on great apes are urgently needed.

It seems probable that cryptic great ape STDs could go undetected for long time periods, even in small, closely monitored populations. As an example, wild chimpanzees have been the subject of intense observational research since the 1960's (Pusey et al. 2007); yet it took several decades to realize that chimpanzees harbor SIV (Peeters et al. 1989). SIV is now one of the best-studied STDs among African apes; SIV prevalence is known to vary greatly (from absent to high prevalence) among chimpanzee and gorilla communities (Neel et al. 2010; Rudicell et al. 2010), and the virus has been shown to be pathogenic and can lead to reduced immunity and premature death for chimpanzees (Keele et al. 2009). Other putative STDs that have been isolated from African apes include Simian T-Lymphotrophic Viruses (e.g., Junglen et al. 2010), herpes viruses (Luebcke et al. 2006, e.g.,), papillomaviruses (e.g., Sundberg et al. 1992), and syphilis (e.g., Lovell et al. 2000). Notably however, a majority of these pathogens were detected in zoo or laboratory apes, with few studies demonstrating population-level prevalence for wild apes (but see: Leendertz et al. 2004). Further, we are not aware of any studies that have screened wild or captive apes for naturally occurring *Trichomonas vaginalis*, *Chlamydia trachomatis*, *Mycoplasma genitalium*, or *Neisseria gonorrhoeae*, even though these pathogens are prevalent in human populations (Holmes et al. 2008) and early experimental work demonstrated that apes and monkeys inoculated with these species develop infections similar to those observed in humans (Brown and Lucas 1973; Hegner 1928; Taylor-Robinson et al. 1981; Tully et al. 1986).

To examine population-level prevalence for putative STDs that are common in human populations but have not yet been studied in (non-human) great apes, we screened urine samples from wild eastern chimpanzees for *Trichomonas* spp. and *Chlamydia* spp. using PCR. To investigate if putative STDs previously detected in captive apes are also present in their wild and sanctuary counterparts, we screened vaginal swabs from sanctuary chimpanzee and sera from wild and orphaned eastern gorillas for papillomavirus and *Treponema pallidum* (syphilis and yaws). We also examined how infection status covaries with biological and

ecological factors, such as sex, age, and season. Based on results of prior modeling and comparative research, we predicted that STD prevalence in females would be higher than in males (Nunn and Altizer 2004; Thrall et al. 2000). We also predicted that older, sexually mature individuals would be more likely to test positive for STDs than young individuals. Lastly, to help distinguish whether infectious agents isolated from urine might be originating from genitalia versus fecal contamination, we tested fecal samples for these same organisms, assuming that a high frequency in fecal samples would point towards fecal-oral transmission and away from sexual transmission.

4.3 Methods

Study sites, populations, and samples

Wild chimpanzees: urine and fecal samples

We collected a total of 393 urine samples from 111 wild Ugandan chimpanzees, representing two separate populations. During 2002 – 2010 we collected 294 urine samples from the wild Kanyawara chimpanzee community ($n = 62$ individuals; demography in Table C.1) in Kibale National Park in Southwestern Uganda (Struhsaker 1997). During 2001 – 2007 we collected 99 urine samples from the Sonso community ($n = 49$ individuals; Table C.2) in Budongo Forest in Western Uganda (Reynolds 2005). We collected urine opportunistically throughout the day, immediately following excretion by the animal. Typically, we used a disposable plastic bag attached to a 2m pole to catch urine from a chimpanzee in a tree (as per: Muller and Wrangham 2004); we subsequently pipetted approximately 2ml of urine into sterile tubes. Occasionally, urine was pipetted off of ground leaves, with care taken to avoid visible contaminants. Urine samples were frozen at - 20°C within 1 – 12 hours of collection. Samples were transported on ice to the United States, where they were stored at - 80°C until

analysis.

To assess if trichomonds detected in urine were a possible STD or were more likely associated with fecal contamination, fecal samples ($n = 70$) were collected non-invasively from a subset of Kibale chimpanzees ($n = 30$ individuals, Table C.1) during Jan 2010 – Aug 2010. Within 12 hours of collection, feces were preserved in RNAlater nucleic acid buffer (Ambion) (as per: Johnston et al. 2010). Samples were transported to the United States, where they were stored at - 80°C until analysis.

Sanctuary chimpanzees: vaginal swabs

We collected vaginal swab samples from 15 adult and subadult female chimpanzees at Ngamba Island Chimpanzee Sanctuary (hereafter, Ngamba). This 100-acre forested island in Lake Victoria, Uganda, houses rescued or confiscated chimpanzees, most of which are wild-born. In collaboration with the Chimpanzee Sanctuary and Wildlife Conservation Trust, we collected vaginal swabs ($n = 15$) from the same sanctuary chimpanzees at Ngamba. Sterile swabs (Copan Diagnostics, Corona, CA) were inserted into the vaginal cavity to a depth about 0.5 cm from the vestibule and gently rotated to enable collection of microbes from the vaginal walls. Duplicate samples from the same animal were collected. Swabs were immediately placed in sterilized screw cap tubes pre-filled with RNAlater (Ambion Cat # 7020); tubes were flash frozen or placed on ice and then transferred to - 80°C freezers until sample processing.

Wild and orphan gorillas: serum samples

We collected serum samples ($n = 46$) from gorillas between 1988 and 2007, which included both wild mountain gorillas (*G. b. beringei*, $n = 40$ gorillas) and eastern lowland gorillas (*G. b. graueri*, $n = 6$ gorillas). Mountain gorilla subjects were located in the Virunga Massif habitat or the Bwindi Impenetrable Forest. Wild-born eastern lowland gorilla orphans were

confiscated by the law enforcement and housed at the Eastern Gorilla Interim Quarantine Facility in Kinigi, Rwanda. Blood samples were opportunistically collected from identified, habituated, wild mountain gorillas during emergency health interventions and from orphaned gorillas during regular health exams (as per: Milligan et al. 2008). Sera were frozen at - 20°C within 6 hours and were shipped on dry ice to the United States where they were stored at - 80°C until analysis. Demographics for gorilla subjects are included in Table C.3.

Molecular methods

DNA extraction

We extracted DNA from chimpanzee urine samples using the DNeasy Blood and Tissue Kit (QIAGEN, Valencia California) with the manufacturer protocol for cultured cells. To extract DNA from chimpanzee fecal samples, we used the QIAamp DNA Stool Mini Kit per the manufacturer's instructions. Lastly, to extract DNA from vaginal swabs, the frozen samples were thawed on ice and homogenized in sterile phosphate buffered saline. Samples were then spun for 5 min at full speed to wash out salts in RNAlater. Subsequent steps included lysozyme incubation (20 mM Tris-HCl at pH 7.4, 100 mM EDTA, 50 mM NaCl, 0.2% Tween), addition of 10% SDS, freeze-thaw cycling, proteinase-K incubation, protein precipitation using 5M NaCl, incubation on ice, and centrifugation, RNAse treatment, followed by phenol-chloroform extraction and alcohol precipitation. To prevent and detect potential contamination, all DNA extraction, primary PCR, secondary PCR, and gel electrophoresis were all conducted in separate laboratory areas and at least one negative control sample was included in each extraction and PCR batch.

PCR assays

Wild chimpanzee urine samples were tested for trichomonads using a nested PCR as described in Felleisen (1997) and following the manufacturer's conditions (Promega, Madison, Wisconsin). Primary amplification used trichomonad-specific primers TFR1 and TFR2, which amplified the ITS1, 5.8S rRNA, and ITS2 regions (Felleisen 1997, Table C.4); primers K1-5.8S-100 and K1-28S-338 were used in secondary reactions (Table C.4). We included samples of *T. vaginalis* and *T. gallinae* in each run as positive controls and separate water negative controls in primary and secondary PCR batches. We visualized amplicons using gel electrophoresis and considered samples positive for trichomonads when bands of $\approx$ 246 bp were present. Following the detection of positive samples, we conducted follow-up PCR with wild chimpanzee fecal samples. Because of the high prevalence using only the primary primers TFR1 and TFR2, no secondary PCR was run on fecal samples. We considered fecal samples with bands of $\approx$ 388 bp to be positive for trichomonads.

To screen wild chimpanzee urine samples for *Chlamydia* spp., we used nested PCR targeting a 142 bp segment of the omp1 gene, which encodes the major outer membrane protein of chlamydiae (Sachse and Hotzel 2003). PCR was set up according to the manufacturer's conditions (Promega, Madison, Wisconsin). For primary amplification, we used primers CG-Omp-1-F and CG-Omp-424-R, and for the secondary amplification, we used primers CG-Omp-78-Fi and CG-Omp-219-Ri (Table C.4). As positive controls, we included samples of *C. psittaci* and *C. trachomatis* in each run, along with negative separate water controls in primary and secondary amplifications. We assessed amplicons with gel electrophoresis and considered samples with 142 bp bands to be positive for *Chlamydia* spp.

To screen sanctuary chimpanzees for papillomaviruses, DNA extracted from vaginal swabs were submitted to a professional diagnostic company (Zoologix Inc., CA, USA), for real time PCR testing designed to detect papillomaviruses in nonhuman primates. Lastly, we screened gorilla serum samples for *Treponema pallidum* using a qualitative rapid plasma reagin (RPR)

test (Inverness Medical, NJ) as per the manufacturer's instructions.

Sequencing and phylogenetic analysis of positive samples

We sequenced a subset of positive trichomonad PCR amplicons from urine samples using an Illumina MiSeq instrument. Amplicons were ligated to Illumina TruSeq style adapters with custom indexes (Faircloth and Glenn 2012) using a protocol derived from Fisher et al. (2011). The resulting libraries were spiked into a pool of genomic libraries unrelated to this research. Because the amplicon libraries were only a small percentage of the overall pool ($<$ 1%), no special accommodations were required. Paired-end 150 base reads were obtained and samples were demultiplexed using Illumina MiSeq software.

For phylogenetic analysis, we aligned paired forward and reverse sequences using Geneious v. 5.5.6 (Drummond et al. 2010). We then clustered redundant sequences with more than 96% overlap. We identified a single representative sequence from each cluster with CD-hit (Li and Godzik 2006). Using the default settings in MAFFT v. 7 (Katoh et al. 2002), we aligned the resulting seven sequence reads from our study with eight *Trichomonas* spp. and *Tetratrichomonas* spp. sequences from the NCBI GenBank database. Bayesian trees were generated in MrBayes v. 3.2.1 (Ronquist and Huelsenbeck 2003) with two independent runs of four chains that each ran for 20 million generations. Trees were sampled every 200 generations, with the first 25% of sampled trees discarded as burn in. Because the model for nucleotide substitution determined by jModelTest (Posada 2008) was not available in MrBayes, we used a mixed model with gamma substitution. We compared tree files obtained from independent runs using AWTY (Nylander et al. 2008) to confirm convergence.

Statistical analysis

To identify biological factors associated with infection status, we used generalized linear models (GLMs) to test for significant relationships between the infection status of an indi-

vidual (1/0), where chimpanzees with at least one positive sample were assigned a 1, and the following predictor variables: sex, age (averaged across sample collection dates), population, the number of samples per individual, and two-way interactions. To further examine ecological and temporal predictors of infection status, we controlled for sample collection year and used generalized linear mixed models (GLMMs) to test the following predictor variables: age at time of sample collection, sex, sample collection season, and two-way interactions. We categorized season as dry (Dec – Feb, Jun – Aug) or rainy (Mar – May, Sept – May) (Hartter et al. 2012; Reynolds 2005). To account for repeated sample collection from some individuals, we assessed model fit with a random effect of individual nested within population.

4.4 Results

All chimpanzee urine samples, chimpanzee vaginal swabs, and gorilla serum samples were negative for *Chlamydia* spp., papillomavirus, and *Treponema pallidum*, respectively (Table 4.1). However, 35.9% of the chimpanzee urine samples tested positive for trichomonads, including 39.5% and 25.3% of the samples from Kibale and Budongo populations, respectively. The status of some individuals transitioned between time points from either positive to negative, or vice versa. Based on individuals, a total of 77.4% of the Kibale population and 36.7% of the Budongo population had at least one positive sample.

GLMs revealed that males were significantly less likely than females to have at least one urine sample positive for trichomonads ($\beta = -1.20$, $P = 0.018$, Table 4.2a, Figure 4.1), and chimpanzees in Kibale were significantly more likely to have at least one positive sample than chimpanzees in Budongo ($\beta = 1.09$, $P = 0.023$, Table 4.2a). Individuals with more samples were significantly more likely to have at least one positive sample ($\beta = 0.68$, $P < 0.001$, Table 4.2a). After controlling for collection year, GLMMs conducted at the level of the sample again showed that urine samples collected from males were less likely to be infected

with *Trichomonas* spp. than samples from females (β = – 0.49, P = 0.032, Table 4.2b). Additionally, samples were significantly more likely to be positive during the dry season (β = – 0.62, P = 0.014, Table 4.2b). Neither age nor any two way-interactions were significant at the individual or sample level.

Table 4.1: Pathogen prevalence by species and population.

Species	Population	n	Habitat[a]	n of each sample type[b]				Pathogen prevalence[c]			
				Urine	Feces	Swab	Serum	Trichomonads	*Chlamydia*	Papillomavirus	*Treponema*
Chimpanzee	Kibale	62	w	294	70	-[e]	-	77.4% (90.3%)[d]	0%	-	-
	Budongo	49	w	99	-	-	-	36.7%	0%	-	-
	Ngamba	15	s	-	-	15	-	-	-	0%	-
Gorilla	All pooled[f]	46	w/s	-	-	-	46	-	-	-	0%

[a] Descriptor for if population was wild (w) or in a sanctuary (s)
[b] The number of samples per population is shown
[c] Percentage of individuals in the population that had at least one positive samples
[d] Pathogen prevalence for feces is shown in parentheses for the Kibale population, with urine shown outside parentheses
[e] (-) means not tested
[f] Gorilla samples were pooled from the following populations: Virunga Massif, Bwindi Impenetrable Forest, and Eastern Gorilla Interim Quarantine Facility

Table 4.2: Model estimates for individual and sample Trichomonadidae infection status.

a. Predictors of individuals with at least one positive sample [a]

	Urine data			Feces data		
Predictor	**Estimate**	**SE**	***P***	**Estimate**	**SE**	***P***
Intercept	-2.00	0.67	**0.003**	18.89	4052.9	0.996
Sex (male)	-1.20	0.51	**0.018**	-19.42	4052.9	0.996
Age	0.03	0.02	0.169	-0.04	0.05	0.475
Number of samples	0.68	0.20	**< 0.001**	1.40	1.11	0.207
Population (Kibale)	1.09	0.48	**0.023**	NA[b]	NA	NA

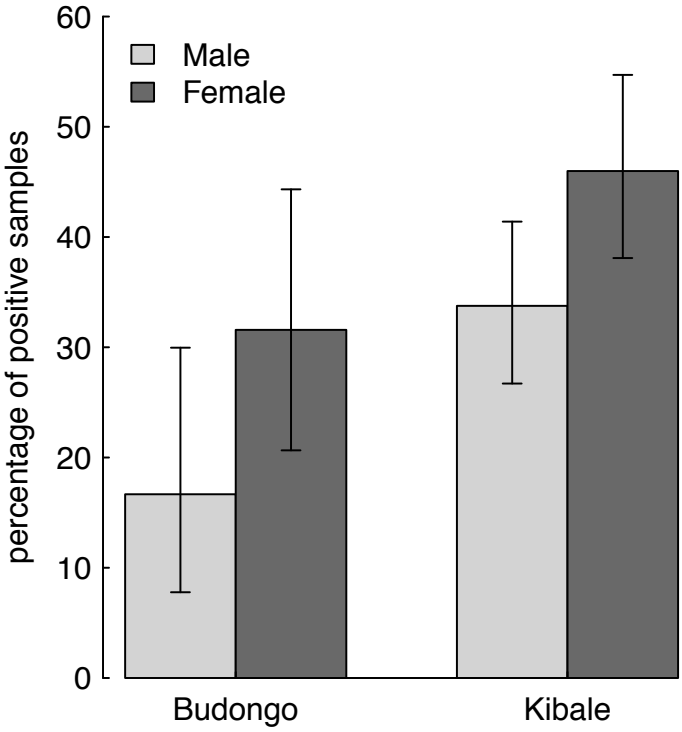
b. Predictors of sample infection status

	Urine data			Feces data		
Predictor	**Estimate**	**SE**	***P***	**Estimate**	**SE**	***P***
Intercept	0.57	0.57	0.315	1.56	0.60	**0.009**
Sex (male)	-0.49	0.23	**0.037**	-0.65	0.59	0.267
Age	0.02	0.01	0.112	-0.04	0.02	0.052
Season (wet)	-0.62	0.25	**0.014**	2.13	0.85	**0.012**

[a] After controlling for year of sample collection (full table in Appendix C: Table C.6)
[b] Population was not included in models for fecal data, as feces were only collected in one population

We found that 68.1% of the fecal samples collected from Kibale chimpanzees were positive for trichomonads, with 90.0% of the individuals screened having at least one positive sample. Similar to data from urine samples, some individuals changed infection status over time. GLMs showed no significant relationships between the likelihood of an individual having at least one fecal sample positive for trichomonads and the following predictors: sex, age and the number of fecal samples collected per individual (Table 4.2a). The lack of significant results is likely an effect of low variance due to extremely high prevalence. At the level of the sample, GLMMs showed that fecal samples were significantly more likely to be infected with trichomonads during the wet season than during the dry season ($\beta = 2.13$, $P = 0.012$, Table 4.2b). Additionally, infection probability decreased with age, with more positive samples

Figure 4.1: Positive trichomonad urine samples broken down by population and sex. The percentages of wild chimpanzee positive urine samples are shown for Budongo and Kibale populations, with light and dark bars representing males and females, respectively. Error bars show 95% confidence intervals.



from younger chimpanzees ($\beta = -0.04$, $P = 0.052$, Table 4.2b), but did not depend on sex.

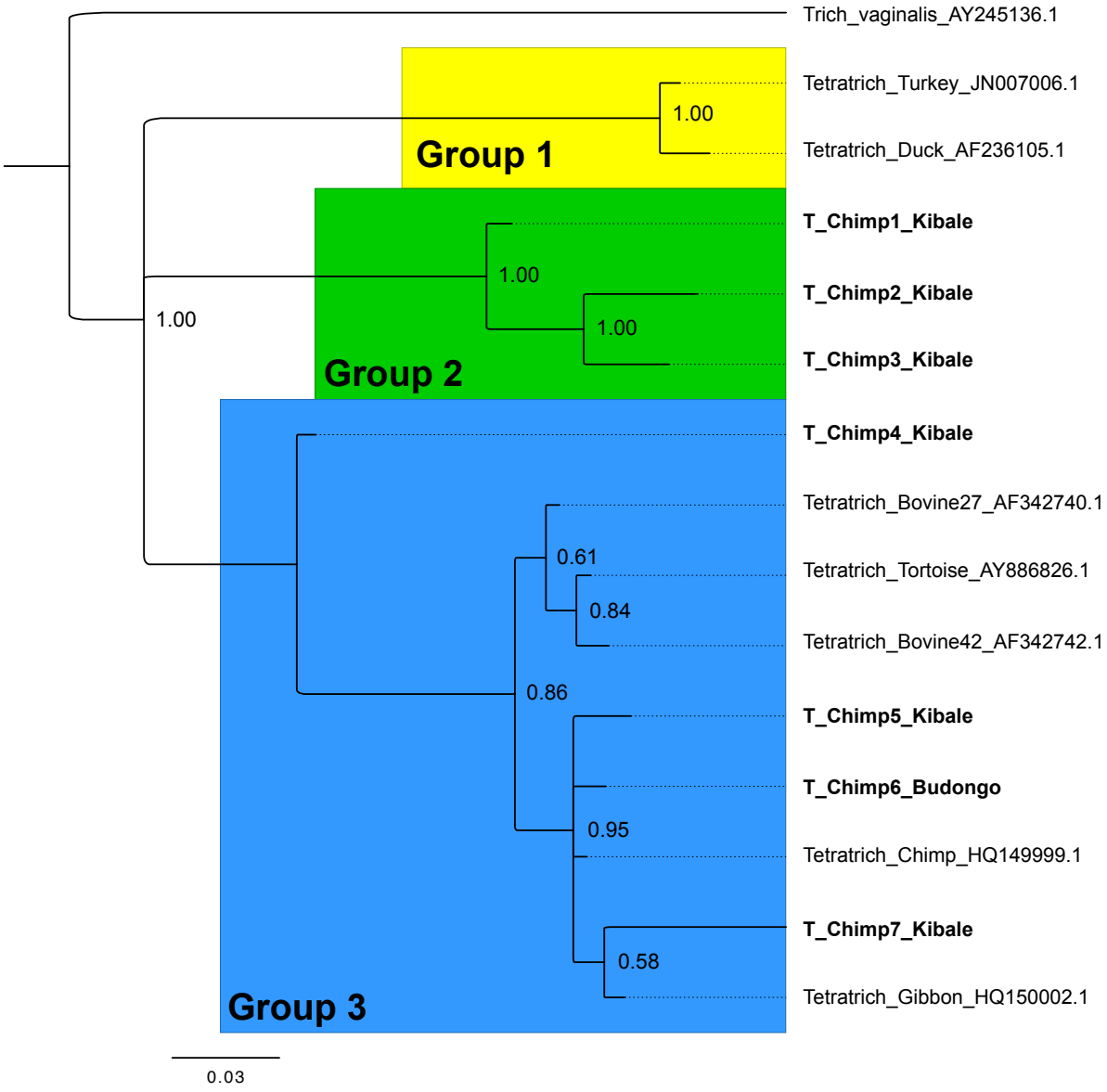
A subset ($n = 16$) of wild chimpanzee urine samples positive for trichomonads were sequenced. When compared to existing sequence data in GenBank, the sequences were most similar to previously described *Tetratrichomonas* spp. Thus, we constructed a phylogenetic tree using our newly generated sequences, previously described *Tetratrichomonas* spp. sequences (Cepicka et al. 2006; Crespo et al. 2001; Reinmann et al. 2012; Smejkalova et al. 2012; Walker et al. 2003) and a *Trichomonas vaginalis* outgroup (Cepicka et al. 2005, Figure 4.2). The tree revealed three major sequence groups (in addition to the *T. vaginalis* root),

with two groups containing chimpanzee sequences. Group 1 included *Tetratrichomonas* spp. from a turkey (i.e., *Tetratrichomonas gallinarum*: undescribed sample type) and a *Tetratrichomonas* spp. isolated from the oviduct of a Pekin duck. Group 2 included only sequences from chimpanzees sampled here, and Group 3 included sequences from chimpanzees in this study in addition to previously described *Tetratrichomonas* spp. isolated from the feces or gastrointestinal tract of cows, tortoises, chimpanzees and gibbons.

4.5 Discussion

Among wild and sanctuary chimpanzees and gorillas examined here, we found no evidence for three out of four unique pathogen groups representing known or suspected STDs. Specifically, wild chimpanzee urine samples, chimpanzee vaginal swabs, and gorilla serum samples were all negative for *Chlamydia* spp., papillomavirus, and *Treponema pallidum*, respectively. This is surprising considering that chlamydia is one of the most prevalent human STDs (Holmes et al. 2008) and syphilis is well documented in other primates, including baboons (Fribourg-Blanc and Mollaret 1969; Harper et al. 2012). We note that due to small sample size, a lack of positive *Treponema pallidum* and papillomavirus results do not necessarily imply absence among all wild ape populations. However, given our large sample size for *Chlamydia* tests, our results indicate that *Chlamydia* spp. are either absent or present at extremely low rates in the two populations we sampled. Despite evidence that other bacteria in the *Chlamydia* genus have zoonotic origins (Myers et al. 2009), little is currently known about the evolution of human-infecting *C. trachomatis* (Clarke 2011). Some human STDs (e.g., HIV/SIV, HTLV/STLV) are thought to have originated in wild primates (Courgnaud et al. 2004; Keele et al. 2006), but our results are not consistent with a shared origin of chlamydia in chimpanzees, although more work is needed to investigate the presence or absence of this pathogen in additional ape populations.

Figure 4.2: A Bayesian phylogeny of positive trichomonad sample sequences. Samples tested here fell into two distinct groups (Groups 2 and 3). Group 1 sequences included a putative STD (Trich_Duck_AF236105.1); Group 2 sequences were only from samples tested here, and Group 3 samples were closely related to *Tetratrichomonas* spp. isolated from feces or gastrointestinal tracts of animals. Phylogenies were constructed using MrBayes v. 3.2.1 and FigTree v. 1.3.1. Numbers on branches indicate Bayesian posterior probabilities. Newly generated sequences are shown in bold as T_Chimp with an arbitrary sequence number and the name of the population from which the sample was collected. Referenced sequences are shown with GenBank accession numbers. The scale bar indicates genetic distance.



In contrast to the other tests, the trichomonad PCR yielded many positive results, with 37% of Budongo chimpanzees and 77% of Kibale chimpanzees having at least one positive urine sample. Follow-up tests revealed that 90% of Kibale chimpanzees had at least one positive fecal sample, indicating that some or all of the positive urine samples could have been contaminated with feces prior to sample collection (e.g., by urine running across fecal particles on hair or skin in the genital region). However, GLMs indicated different predictors of infection status for pathogens isolated from urine and fecal samples, suggesting they could be different pathogens or that they have different transmission routes. In particular, females were significantly more likely than males to have positive urine samples, whereas there was no sex effect for fecal sample infection status. There was no effect of age on the infection status of urine samples, whereas younger chimpanzees were more likely to have positive fecal samples. Lastly, urine samples were more likely to be positive during the dry season, whereas fecal samples were more likely to be positive during rainy months.

Sequencing revealed that the positive chimpanzee urine samples were genetically similar to previously detected *Tetratrichomonas* spp. The genus *Tetratrichomonas*, like *Trichomonas*, belongs to the family Trichomonadidae. To our knowledge, this is the first description of tetratrichomonads in wild chimpanzees; however several tetratrichomonads have recently been reported from captive, wild, and domestic animal hosts, including some captive apes (Cepicka et al. 2006; Crespo et al. 2001; Reinmann et al. 2012; Smejkalova et al. 2012; Walker et al. 2003). Phylogenetic analysis of eight recently reported tetratrichomad sequences along with our newly generated sequences revealed two main sequence groups from chimpanzees, one of which contained only newly generated sequences from urine samples in our study, and a second of which contained both urine sample sequences from our study and previously described sequences from feces or the cecum of mammals and reptiles (e.g., Cepicka et al. 2006). Most previously described tetratrichomonads are believed to be transmitted via fecal-oral routes (Cepicka et al. 2006, 2005), although the duck tetratrichomonad

could be sexually transmitted (Crespo et al. 2001). *Tetratrichomonas* are not generally considered to be pathogenic; however, rare cases of morbidity or mortality do occur (Laing et al. 2013; Mantini et al. 2009).

It is not yet clear how the chimpanzee *Tetratrichomonas* are transmitted, as our findings offer evidence for both sexual and fecal-oral transmission routes. The increased infection rate in female versus male urine samples is characteristic of STDs in polygynous mating systems (Nunn and Altizer 2004; Thrall et al. 2000). On the other hand, the sex difference could result from anatomical factors, as urine collected from females might more commonly contact feces than urine collected from males. Also, some urine sequences were highly similar to tetratrichomonads thought to spread via fecal-oral routes, and the lack of an age effect on urine sample infection status suggests fecal-oral over sexual transmission (although play mating, which starts at a very young age in chimpanzees, could allow for STD transmission prior to sexual maturity). Thus, while some urine sequences (those in Group 3) are likely transmitted via feces, other urine sequences (e.g., Group 2) could be sexually transmitted. More work, including sequencing and comparison of Trichomonadidae isolates from positive fecal samples, is needed to better clarify the transmission routes of these isolates.

Increasing awareness and knowledge of great ape STDs could contribute crucial information for great ape conservation and management. Animal reintroduction, in which captive animals are released into the wild, is a conservation tool currently used for great apes (Farmer and Courage 2008; Tutin et al. 2001); yet, to maintain healthy wild populations, the success of these reintroductions relies on the release of healthy individuals. With limited knowledge of ape STDs, comprehensive STD panels cannot currently be performed prior to release. Identifying which STDs might negatively affect host fitness could greatly improve reintroduction project screening. Similarly, understanding the distribution of STDs in wild ape populations could provide important insights for disease management, particularly if those pathogens lower fecundity (e.g., Keele et al. 2009). Lastly, more extensive data on great

ape STDs could point towards the evolutionary origins of human STDs (beyond our current knowledge of SIV/HIV, STLV/HTLV: Courgnaud et al. 2004; Keele et al. 2009, 2006), and might even provide insights towards novel resistance traits.

Future work on STDs in apes could focus on culturing the *Tetratrichomonas* organisms detected in the current study and on investigating its pathogenicity and transmission routes. Other work might also explore evidence for STDs in bonobos, which demonstrate extreme promiscuity (Kano 1992) and thus might support multiple STDs (Nunn and Altizer 2006). Lastly, researchers should screen apes for additional putative STDs, including *Mycoplasma genitalium* or *Neisseria gonorrhoeae*, two pathogens that are highly prevalent in human populations (Holmes et al. 2008) and can cause infection in apes and monkeys after experimental inoculation (Brown and Lucas 1973; Tully et al. 1986). In conclusion, while our study provided little evidence of STDs in wild and sanctuary apes, we argue that additional work focused on this subject will provide invaluable information for the management of endangered great ape species and for our knowledge of human STD origins.

4.6 Acknowledgements

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Island Chimpanzee Sanctuary staff (Joshua Rokundo, Lilly Ajarova) helped with sample collection for sanctuary chimpanzees.

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Chapter 5

CONCLUSIONS

Because pathogen-induced population declines can threaten wild ape persistence, understanding drivers of pathogen transmission in great apes is an important conservation concern. By incorporating innovative field and computational approaches, this research represents the first network analysis to examine infectious disease dynamics in wild apes. Work described here lends support to the prediction that social and ecological factors can profoundly affect host-parasite dynamics in free-living populations. Modeling techniques developed here could be applied to a broad range of social animals for which contact network data or individual trait data are available. Importantly, this research offers insights for control measures by illustrating how a better understanding of contact heterogeneity and its effect on pathogen outbreaks can help to focus vaccination programs. In particular, results from this work indicate that imperfect vaccination coverage targeted at highly connected individuals is considerably more effective than random control efforts. Although the STD survey of wild and sanctuary apes conducted here did not provide strong evidence for sexually transmitted pathogens, we described for the first time a new tetratrichomonad infecting wild apes. In Summer 2013, I will return to Uganda to share results with veterinarians and wildlife management planners. It is my hope that findings from this research will be incorporated into disease control strategies for habituated wild ape populations.

Appendix A

SUPPORTING INFORMATION FOR CHAPTER 2

A.1 Supplementary Text

A.1.1: Additional information on the Kanyawara chimpanzee community

The Kanywara chimpanzee community was partially habituated to human presence by M. Ghiglieri during 1979-1980 and G. Isabirye-Basuta during 1983-1985. R. Wrangham founded the Kibale Chimpanzee Project (KCP) in 1987. Since this date, Wrangham and colleagues have continuously collected systematic data (e.g., including information on life history, behavioral development, and social relationships) on the Kanyawara chimpanzees, which have been habituated to human presence since 1990 (Wrangham et al. 1992). KCP, including a site manager and five full-time field assistants, is currently funded by grants from the U.S. National Science Foundation (awards 9807448 and 0416125), the U.S. National Institutes of Health, National Geographic Society, L.S.B. Leakey Foundation, and the Wenner-Gren Foundation. To collect behavioral data for this paper, J. Rushmore worked alongside and received assistance from KCP researchers and field assistants.

A.1.2: Rank categorization of study subjects

Chimpanzees were assigned to one of seven coarse rank categories (for adult males: M1, M2, or M3; for adult females: F1 or F2, and for juveniles: J1 or J2) where lower numbers indicate higher ranks. Females were categorized as core-ranging (higher rank F1) or edge-ranging (lower rank F2) according to Kahlenberg et al. (2008) with one exception. BL, a female that Kahlenberg et al. (2008) categorized as core-ranging, was typically found in the southern (i.e., periphery) areas of the Kanyawara range during our study period. Because female range can change over time, we conducted all data analyses twice: first with BL categorized as a core female and again with BL categorized as an edge female. Results were

consistent regardless of how BL was categorized; however, scoring BL as an edge female (lower ranking) consistently increased model fit (as demonstrated by R^2 and DIC values). Thus, for the present study, we considered BL to be an edge female. Juveniles were assigned to the same range areas as their mothers (i.e., ranks J1 or J2, where J1 corresponds to the juvenile offspring of a higher ranking mother).

Adult males were grouped into three rank categories: high (M1)-, medium (M2)-, or low (M3)-rank. To assign males to rank categories, we asked each of six field assistants who had observed the Kanyawara chimpanzee community for 1 – 17+ years to score the 12 adult males in a linear hierarchy from highest- to lowest-rank at the start and end of the study period based on cumulative observations of pant-grunt vocalizations and outcomes of agonistic interactions (after Wittig and Boesch 2003). We then calculated the average linear hierarchy score assignment for each adult male at the start and end of the study. By assigning five males to the high-ranking category (M1), four males to the medium-ranking category (M2), and three males to the low-ranking category (M3), we developed a ranking system in which every male stayed within the same rank category throughout the entire study period (despite minor reshuffling in the linear hierarchy over the nine-month study duration).

A.1.3: Supplementary information on statistical analyses

Quadratic Assignment Procedure

The quadratic assignment procedure (QAP) calculates a Pearson's correlation coefficient for corresponding cells in two observed matrices and then re-calculates the correlation coefficient after the rows and corresponding columns of one of the matrices are randomly permuted (Baker and Hubert 1981). In our analyses, this process was repeated 30,000 times to calculate a P-value.

Bayesian Logistic Mixed Effects Models

When fitting models to our data, we used uninformative priors, which we tested with sensitivity analyses following Kéry (2010), and we inspected goodness of fit across models by calculating the deviance information criterion (DIC) (Kéry 2010). During model selection we removed non-significant terms in favor of the most parsimonious model with the best fit (as demonstrated by low DIC). We ran the models for 300,000 iterations with a burn-in of 25,000 iterations and a sampling regime (i.e., thin) of 15.

Node-level regression

Node-level permutation-based regression is a valuable statistical test for network analysis because it accounts for the fact that network nodes are not independent (Hanneman and Riddle 2005). The test first calculates the regression slope coefficients of the observed dataset; then, over a large number of permutations, the algorithm randomly shuffles values of the dependent variable among network nodes while leaving the values of the independent variables in place. Regression coefficients are recalculated after each permutation, and the P-value for each parameter is calculated by determining the proportion of permutations that yielded values as or more extreme than the original regression for the observed dataset.

A.1.4: Discussion of how fruit availability or presence of sick chimpanzees might affect network structure

Past studies at some field sites showed that chimpanzee party sizes increase with food availability (e.g., Wrangham 2000) whereas no or limited effects of food were seen at other sites (Hashimoto et al. 2003; Newton-Fisher et al. 2000; Reynolds 2005). Given our limited data on fruit availability, we were unable to fully explore how fruiting patterns affect network dynamics. Additionally, little is currently known about how the presence of sick chimpanzees might

affect network structure. During the study duration, we occasionally observed chimpanzees showing outward signs of respiratory infections (e.g., sneezing, coughing), but we did not observe any major respiratory outbreaks (e.g., Köndgen et al. 2008; Williams et al. 2008). While anecdotal evidence suggests that primates can sometimes identify sick community members, there is little indication in the primate literature that individuals self-quarantine or avoid sick conspecifics (Nunn and Altizer 2006). Thus, understanding how network dynamics might be affected by fruiting patterns or the presence of sick individuals remain important areas for future research.

A.2 Supplementary Tables

Table A.1: Stability of networks across two-week to month time steps. For each month, a) party association indices (PAIs) and b) 5m association indices (5mAIs) calculated for weeks 1 – 2 and weeks 3 – 4 were compared to the respective association indices calculated during the entire month. Correlation values (r) and probabilities (P) are based on a quadratic assignment procedure with 30,000 permutations. All correlations are significant with $P < 0.001$. See Appendix Text A.1.3 for additional analysis details.

		Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
a. PAIs										
Weeks 1-2	*r*	0.395	0.787	0.921	0.867	0.900	0.976	0.986	0.949	1.000
	P	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Weeks 3-4	*r*	0.955	0.979	0.818	0.952	0.967	0.852	0.963	0.568	
	P	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	
b. 5mAIs										
Weeks 1-2	*r*	0.675	0.584	0.845	0.922	0.132	0.878	0.851	0.822	1.00
	P	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*0.019*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Weeks 3-4	*r*	0.775	0.849	0.816	0.860	0.835	0.645	0.490	0.859	
	P	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	

Table A.2: Effect of social factors on pairwise associations (PAIs) in party networks using two-week time steps. The posterior mean, 95% credible interval, P-value based on MCMC sampling, and odds ratios (OR) are shown for fixed effect parameters. Bolded relationships are significant at $P < 0.05$. Sex/estrus and age categories are abbreviated as follows: age (adult: adult, AA; adult: juvenile, AJ; juvenile: juvenile, JJ), sex/estrous (pairwise combinations of male (M), female in estrus (Fe) and female not in estrus (F)). Baseline age (AA) and sex/estrus (F:F) are not shown. MCMC simulations were run for 300,000 iterations with a burn-in of 25,000 iterations and a sampling regime of 15. The main difference between analyses conducted at two-week and monthly time steps is that with the two-week data set, there was not a significant interaction between the number of estrous females per month and age, whereas this interaction was significant with the monthly time scale (Table 2.1). All other results were consistent across time steps.

Factor	Posterior Mean	95% CI	*P*	OR
Intercept	-3.88	(-5.4, -2.29)	**<0.001**	
Related	3.55	(3.15, 3.95)	**<0.001**	34.83
Sex (M:F)	0.78	(-0.37, 1.73)	0.162	2.18
Sex (M:M)	1.32	(-0.75, 3.38)	0.226	3.74
Sex (F:Fe)	3.01	(2.48, 3.59)	**<0.001**	20.2
Sex (M:Fe)	4.16	(2.8, 5.21)	**<0.001**	63.8
Difference in family size	-0.16	(-0.23, -0.08)	**<0.001**	0.86
Difference in rank	-1.29	(-1.48, -1.12)	**<0.001**	0.27
Age (AJ)	0.65	(-0.33, 1.77)	0.212	1.92
Age (JJ)	0.91	(-1.2, 2.9)	0.358	2.49
Number (#) of estrous females	1.00	(0.82, 1.16)	**<0.001**	2.71
# of estrous females:Age (AJ)	-0.16	(-0.38, 0.06)	0.168	2.31
# of estrous females:Age (JJ)	-0.26	(-0.61, 0.05)	0.134	2.09

Table A.3: Effect of social factors on party and 5m-association network centrality measures using two-week time steps. Coefficients (β) and P-values are presented. Bolded values indicate significant relationships after Bonferroni correction. R^2 values are shown for each test; baseline rank (M1) and time interval (Apr: Wk 1 – 2) categories are not shown. Results are consistent between two-week and monthly time steps (Table 2.2) in that family size and rank were the most important predictors for individual centrality at both the party and 5m-level after controlling for the time interval of observation (i.e., the first and second two weeks of a month: Wk 1 – 2 and Wk 3 – 4, respectively). Analyses at both time steps indicated that core-ranging females and juveniles (F1 and J1) with large families and to a lesser extent high-ranking males (M1) were significantly more central than other community members.

*Table shown on next page.

Table A.3 (continued)

	Party association networks, N=492						5m association networks, N=492					
	Degree		Eigenvector		Flow-betweenness		Degree		Eigenvector		Flow-betweenness	
	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*
Intercept	20.62	**<0.001**	0.18	0.333	30.5	0.148	-0.05	**<0.001**	0.04	**0.010**	3.64	**<0.001**
Rank: M2	-0.51	0.322	-0.01	0.324	5.46	**0.010**	0.01	0.449	-0.02	0.245	6.22	0.094
Rank: M3	-1.32	0.124	-0.02	0.093	0.30	0.418	-0.18	**0.004**	-0.08	**0.004**	0.38	0.451
Rank: F1	-0.67	0.261	-0.01	0.208	1.63	0.204	0.01	0.428	-0.03	0.155	-0.50	0.459
Rank: J1	-0.18	0.431	-0.01	0.329	1.78	0.194	-0.05	0.244	-0.04	0.098	-0.43	0.460
Rank: F2	-4.24	**<0.001**	-0.07	**<0.001**	-2.72	0.092	-0.15	**0.014**	-0.06	**0.013**	-2.37	0.317
Rank: J2	-3.96	**0.001**	-0.06	**<0.001**	-0.64	0.412	-0.09	0.110	-0.05	0.075	6.38	0.123
Estrus	1.41	0.231	0.05	0.024	-1.99	0.259	0.09	0.213	0.09	0.060	-2.30	0.439
Family size	0.540	0.026	0.01	**0.008**	-0.58	0.144	0.07	**<0.001**	0.02	**0.001**	-0.74	0.266
Dec												
Wk 1-2	-18.20	**<0.001**	0.03	0.070	-14.32	**<0.001**	0.56	**<0.001**	0.05	0.155	14.25	0.036
Wk 3-4	-10.50	**<0.001**	-0.01	0.246	-6.21	0.029	0.43	**<0.001**	0.05	0.102	19.29	**0.003**
Jan												
Wk 1-2	-16.45	**<0.001**	-0.01	0.289	-4.82	0.050	0.32	**<0.001**	0.03	0.207	17.24	**0.006**
Wk 3-4	-14.73	**<0.001**	-0.01	0.250	0.69	0.373	0.48	**<0.001**	0.02	0.276	29.24	**<0.001**
Feb												
Wk 1-2	-11.53	**<0.001**	0.00	0.411	-1.27	0.284	0.39	**<0.001**	0.01	0.439	26.69	**<0.001**
Wk 3-4	-15.80	**<0.001**	-0.01	0.279	-7.8	**0.012**	0.48	**<0.001**	0.08	0.036	15.41	**0.015**
Mar												
Wk 1-2	-15.22	**<0.001**	0.00	0.445	-8.91	**0.005**	0.32	**0.001**	0.06	0.085	16.4	**0.012**
Wk 3-4	-12.80	**<0.001**	0.00	0.452	-4.16	0.064	0.24	**0.005**	0.07	0.046	23.57	**<0.001**
Apr												
Wk 3-4	-4.92	**<0.001**	0.00	0.439	1.18	0.290	0.12	0.093	0.08	0.022	27.59	**<0.001**
May												
Wk 1-2	-10.52	**<0.001**	0.00	0.395	1.36	0.266	0.34	**<0.001**	0.06	0.045	26.67	**<0.001**
Wk 3-4	-8.90	**<0.001**	0.00	0.433	-1.93	0.195	0.15	0.050	0.07	0.034	26.64	**<0.001**
Jun												
Wk 1-2	-8.19	**<0.001**	-0.02	0.148	0.84	0.346	0.27	**0.001**	0.08	0.018	26.79	**<0.001**
Wk 3-4	-0.44	0.390	0.01	0.348	-5.2	0.044	0.09	0.159	0.11	**0.004**	20.71	**0.002**
Jul												
Wk 1-2	-7.64	**<0.001**	-0.01	0.327	-0.97	0.333	0.34	**<0.001**	0.09	**0.009**	19.86	**0.002**
Wk 3-4	-15.78	**<0.001**	-0.05	**0.004**	7.04	0.036	0.99	**<0.001**	0.05	0.135	26.95	**<0.001**
Aug												
Wk 1-2	-9.33	**<0.001**	-0.02	0.085	2.98	0.107	0.26	**0.001**	0.03	0.217	28.38	**<0.001**
R^2	0.77	**<0.001**	0.16	**<0.001**	0.18	**<0.001**	0.44	**<0.001**	0.09	**0.006**	0.11	**<0.001**

Table A.4: Individual trait data for study subjects ($n = 37$). This table shows the sex, age class, family size, and rank category for all chimpanzees included in the study.

Chimpanzee ID	Sex[1]	Age Class[2]	Family Size	Rank Category[3]
AJ	M	A	1	M1
AL	F	A	3	F1
AT	M	J	3	J1
AZ	M	J	3	J1
BB	M	A	1	M1
BL	F	A	3	F2
BO	M	J	3	J2
BU	F	J	3	J2
ES	M	A	2[4]	M2
EU	F	J	2[4]	J1
KK	M	A	1	M1
LK	M	A	1	M1
LR	F	A	1	F1
ML	F	A	1	F2
MS	M	A	1	M1
MU	F	A	2	F2
MX	M	J	2	J2
NP	F	J	1	J1
OG	M	J	4	J1
OM	F	J	4	J1
OT	F	J	4	J1
OU	F	A	4	F1
PB	M	A	1	M3
PG	M	A	1	M2
QT	F	A	1	F1
RD	F	A	1	F2
ST	M	A	1	M2
TG	F	A	4	F1
TJ	M	A	4	M3
TS	F	J	4	J1
TT	M	J	4	J1
TU	M	A	1	M2
UM	F	A	2	F2
UN	M	J	2	J2
WA	F	A	1	F2
WL	F	A	1	F1
YB	M	A	1	M3

[1]Sex: M = male, F = female

[2]Age Class: A = adult, J = juvenile (as defined in the main text)

[3]Rank Category: M1, M2, and M3 refer to high-, medium-, and low-ranking adult males, respectively; F1 and J1 refer to core-area adult females and juveniles, respectively; F2 and J2 refer to edge-area adult females and juveniles, respectively

[4]As described in the main text, even though their mother was deceased, ES and EU (a brother-sister pair) were considered a family unit, as ES and EU spent more than 65% of their time together

Table A.5: Effect of social factors on within-party association indices (WPAIs) with best-fit model using monthly time steps. Posterior means (β) and 95% credible intervals (CI) are shown for fixed effect parameters in each of the monthly networks. Sex/estrus and age categories are abbreviated as follows: age (adult: adult, AA; adult: juvenile, AJ; juvenile: juvenile, JJ), sex/estrous (pairwise combinations of male (M), female in estrus (Fe) and female not in estrus (F)). Baseline age (AA) and sex/estrus (FF) are not shown. The results are broken down by month because there was a month interaction with every other parameter. NAs exist in months where no females were observed in estrus, and thus the effect of a pair with an estrous female could not be considered. MCMC simulations were run for 300,000 iterations with a burn-in of 25,000 iterations and a sampling regime of 15. Bolded relationships are significant at the 0.05 level.

*Table shown on next page.

Table A.5 (continued)

		Intercept	Related	Age (AJ)	(JJ)	No Estrus Sex (M:F)	Sex (M:M)	Estrus Sex (F:EF)	Sex (M:EF)	Family Size Diff	Rank Diff
Dec	β	**-0.61**	**1.27**	-0.23	0.03	-0.22	-0.19	NA	NA	-0.59	-0.36
	CI	**(-1.14, -0.10)**	**(0.76, 1.77)**	(-0.64, 0.16)	(-0.64, 0.67)	(-0.69, 0.24)	(-0.83, 0.43)			**(-0.74, -0.45)**	(-0.74, 0.02)
Jan	β	**-0.89**	**1.08**	0.03	-0.30	0.37	**0.72**	-0.20	0.07	-0.03	**-0.42**
	CI	**(-1.4, -0.40)**	**(0.63, 1.53)**	(-0.31, 0.36)	(-0.89, 0.28)	(-0.04, 0.78)	**(0.15, 1.27)**	(-1.41, 0.92)	(-0.75, 0.89)	(-0.13, 0.07)	**(-0.71, -0.13)**
Feb	β	**-1.56**	**1.60**	-0.22	-0.16	0.04	0.45	NA	NA	-0.06	-0.04
	CI	**(-2.02, -1.10)**	**(1.2, 2.00)**	(-0.53, 0.08)	(-0.72, 0.37)	(-0.33, 0.40)	(-0.09, 0.97)			(-0.16, 0.03)	(-0.31, 0.23)
Mar	β	**-2.29**	**1.59**	-0.24	-0.21	0.20	**0.76**	NA	NA	0.09	**-0.51**
	CI	**(-2.77, -1.83)**	**(1.17, 2.00)**	(-0.57, 0.08)	(-0.78, 0.34)	(-0.17, 0.56)	**(0.22, 1.30)**			(-0.01, 0.19)	**(-0.79, -0.24)**
Apr	β	**-3.37**	0.53	**-0.54**	-0.53	0.21	0.43	-0.03	0.52	**-0.41**	**-0.87**
	CI	**(-2.28, -1.11)**	(-0.19, 1.21)	**(-0.96, -0.13)**	(-1.23, 0.15)	(-0.31, 0.72)	(-0.22, 1.09)	(-0.9, 0.8)	(-0.27, 1.3)	**(-0.58, -0.23)**	**(-1.25, -0.50)**
May	β	**-1.54**	**0.75**	0.17	0.48	-0.08	0.27	0.10	-0.55	**-0.11**	-0.80
	CI	**(-1.99, -1.10)**	**(0.33, 1.16)**	(-0.14, 0.47)	(-0.06, 1.01)	(-0.42, 0.25)	(-0.25, 0.78)	(-0.45, 0.63)	(-1.18, 0.06)	**(-0.21, -0.02)**	(-1.04, -0.57)
Jun	β	**-2.16**	**1.14**	**-0.42**	**-0.65**	0.10	**0.71**	-0.73	0.24	-0.01	**-0.51**
	CI	**(-2.66, -1.68)**	**(0.68, 1.59)**	**(-0.74, -0.10)**	**(-1.23, -0.08)**	(-0.31, 0.50)	**(0.15, 1.26)**	(-1.50, 0.00)	(-0.44, 0.9)	(-0.11, 0.08)	**(-0.8, -0.24)**
Jul	β	**-1.45**	**1.41**	**-0.37**	**-0.73**	0.10	**0.58**	**-0.90**	-0.20	-0.03	**-0.87**
	CI	**(-1.92, -0.99)**	**(0.99, 1.82)**	**(-0.7, -0.06)**	**(-1.31, -0.16)**	(-0.28, 0.48)	**(0.03, 1.11)**	**(-1.61, -0.22)**	(-0.79, 0.38)	(-0.13, 0.06)	**(-1.14, -0.60)**
Aug	β	**-1.21**	**1.13**	**-0.37**	-0.40	**-0.96**	-0.02	**-2.83**	-0.47	**-0.29**	**-0.57**
	CI	**(-1.73, -0.71)**	**(0.58, 1.65)**	**(-0.74, -0.01)**	(-0.99, 0.18)	**(-1.39, -0.54)**	(-0.59, 0.54)	**(-4.15, -1.73)**	(-1.12, 0.15)	**(-0.43, -0.15)**	**(-0.9, -0.26)**

Table A.6: P-values for post-hoc tests of rank and centrality in monthly party and 5m-association networks. Bolded values indicate significant relationships after Bonferroni correction. There were no significant differences among rank categories in 5m flowbetweenness tests (Table 2.2).

	Party association networks							5m association networks					
	Rank	M2	M3	F1	J1	F2	J2	M2	M3	F1	J1	F2	J2
Degree	M1	0.108	0.112	0.150	0.243	**<0.001**	**<0.001**	0.153	**0.004**	0.209	0.112	**<0.001**	**<0.001**
	M2		0.472	0.422	0.307	**0.001**	**0.011**		0.051	0.415	0.418	**<0.001**	**<0.001**
	M3			0.388	0.272	**0.001**	**0.015**			0.025	0.056	**0.012**	0.032
	F1				0.347	**<0.001**	**0.002**				0.290	**<0.001**	**<0.001**
	J1					**<0.001**	**<0.001**					**<0.001**	**<0.001**
	F2						0.253						0.391
Eigenvector	M1	0.052	0.078	0.230	0.389	**<0.001**	**<0.001**	0.085	0.028	0.271	0.221	**<0.001**	**<0.001**
	M2		0.474	0.192	0.102	**<0.001**	**<0.001**		0.264	0.226	0.287	**<0.001**	**0.001**
	M3			0.217	0.110	**<0.001**	**0.001**			0.077	0.096	**0.007**	**0.010**
	F1				0.289	**<0.001**	**<0.001**				0.411	**<0.001**	**<0.001**
	J1					**<0.001**	**<0.001**					**<0.001**	**<0.001**
	F2						0.382						0.472
Flowbetweenness	M1	0.212	0.135	0.023	0.019	**<0.001**	**0.002**						
	M2		0.361	0.119	0.097	**0.001**	**0.014**						
	M3			0.217	0.176	**0.007**	0.035						
	F1				0.434	0.018	0.093						
	J1					0.026	0.105						
	F2						0.280						

Table A.7: Effect of social factors on party and 5m-association monthly network centrality measures (full table). Coefficients (β) and P-values are presented. Bolded values indicate significant relationships after Bonferroni correction. R^2 values are shown for each test. Baseline rank (M1) and month (Apr) categories are not shown; P-values for rank post-hoc significance tests are located in Table A.6.

	Party association networks, N=294						5m association networks, N=294					
	Degree		Eigenvector		Flow-betweenness		Degree		Eigenvector		Flow-betweenness	
	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*
Intercept	17.36	**<0.001**	0.17	0.269	33.41	0.058	1.35	0.096	0.13	0.324	35.52	0.109
Rank: M2	-1.43	0.108	-0.03	0.052	-1.41	0.212	-0.23	0.153	-0.04	0.085	-0.75	0.449
Rank: M3	-1.54	0.112	-0.02	0.078	-2.11	0.135	-0.65	**0.004**	-0.05	0.028	-0.15	0.496
Rank: F1	-1.19	0.150	-0.01	0.230	-3.51	0.023	-0.18	0.209	-0.02	0.271	-5.08	0.163
Rank: J1	-0.82	0.253	0.00	0.389	-3.74	0.019	-0.28	0.112	-0.02	0.221	-3.49	0.255
Rank: F2	-5.30	**<0.001**	-0.09	**<0.001**	-6.91	**<0.001**	-1.20	**<0.001**	-0.12	**<0.001**	-7.43	0.074
Rank: J2	-4.47	**<0.001**	-0.08	**<0.001**	-5.82	**0.002**	-1.13	**<0.001**	-0.13	**<0.001**	1.57	0.389
Estrus	0.85	0.324	0.01	0.404	0.24	0.443	0.30	0.204	0.05	0.102	3.72	0.272
Family size	0.73	**0.008**	0.01	**<0.001**	0.57	0.114	0.18	**0.001**	0.02	**0.001**	-0.50	0.356
Dec	-8.93	**<0.001**	-0.02	0.154	-6.06	**0.001**	0.49	0.025	-0.01	0.418	-9.18	0.057
Jan	-11.57	**<0.001**	-0.01	0.252	-0.44	0.399	1.00	**<0.001**	0.02	0.216	0.25	0.481
Feb	-10.00	**<0.001**	-0.01	0.348	-2.53	0.087	0.66	**0.003**	0.04	0.102	-2.59	0.318
Mar	-10.68	**<0.001**	-0.01	0.260	-4.51	**0.009**	-0.26	0.140	0.02	0.227	-6.54	0.122
May	-6.73	**<0.001**	0.00	0.443	0.15	0.463	0.51	**0.016**	0.02	0.272	-0.24	0.484
Jun	-3.30	**0.003**	-0.02	0.135	-0.17	0.465	0.15	0.266	0.01	0.294	-2.38	0.332
Jul	-6.58	**<0.001**	-0.03	0.046	3.42	0.027	0.52	**0.012**	0.02	0.275	-4.22	0.214
Aug	-5.84	**<0.001**	-0.02	0.095	1.88	0.114	-0.06	0.391	-0.02	0.198	-0.69	0.445
R^2	0.618	**<0.001**	0.347	**<0.001**	0.195	**<0.001**	0.406	**<0.001**	0.251	**<0.001**	0.037	0.835

A.3 Supplementary Figures

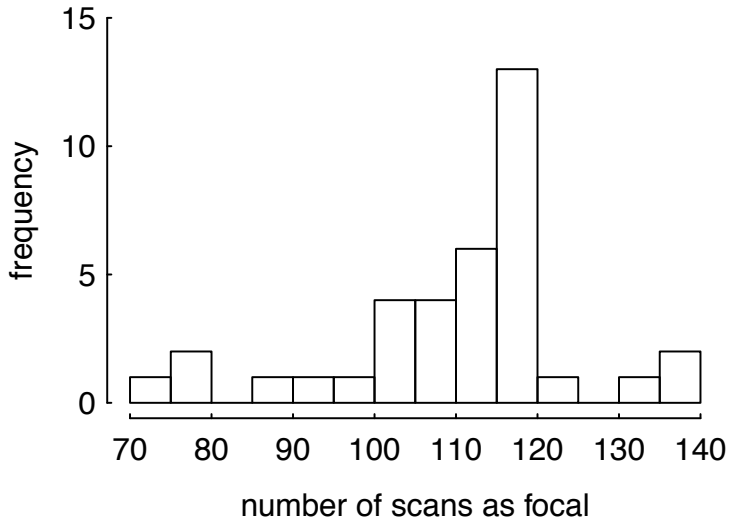

Figure A.1: Histogram of observation effort across individuals. The frequency of individuals is plotted against the number of scans in which an individual was the focal subject. Notably, calculations of weighted centrality measures were based on association indices, which account for observation effort across individuals (see equation 2.1). However, to ensure that sampling effort did not drive centrality measures, we tested for correlations between the number of scans in which an individual was the focal subject and each of the three centrality measures for the individual. As expected, we found no correlations: degree centrality, $R^2 = 0.008$; eigenvector centrality, $R^2 = 0.018$; flow-betweenness centrality, $R^2 = 0.004$.

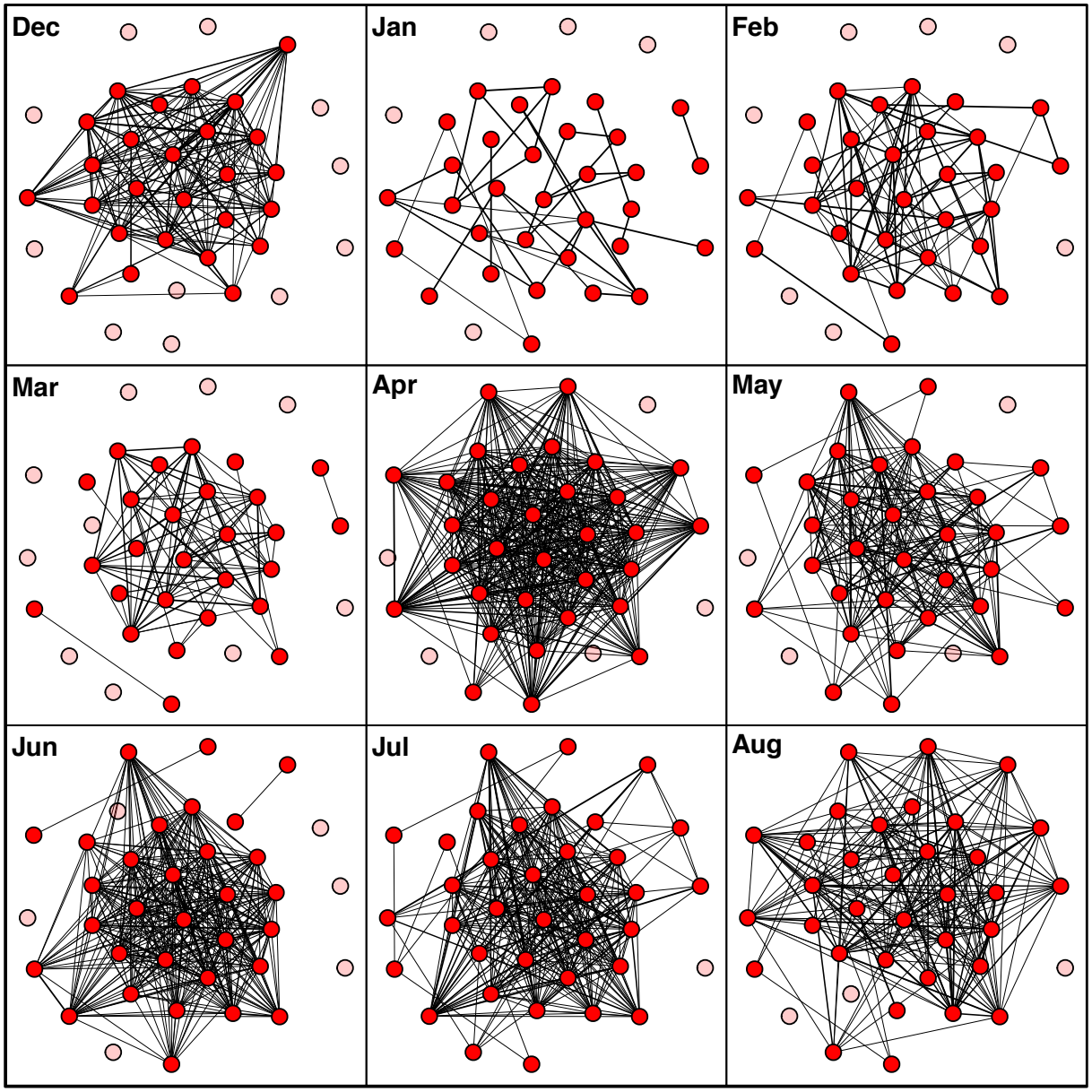

Figure A.2: Monthly party association networks. Nodes (circles) represent individual chimpanzees ($n = 37$) and edges (lines) represent observed associations, where edge thickness corresponds to the pairwise party association index (PAI) between nodes. All networks are displayed with identical layouts and only edges with PAIs > 0.35 are shown. Node color represents connectedness, where dark red nodes have at least one edge above the PAI cutoff and light red nodes do not have any edges above the PAI cutoff. Networks were constructed in R with the igraph package version 0.5.5-4 (Csardi and Nepusz 2006).

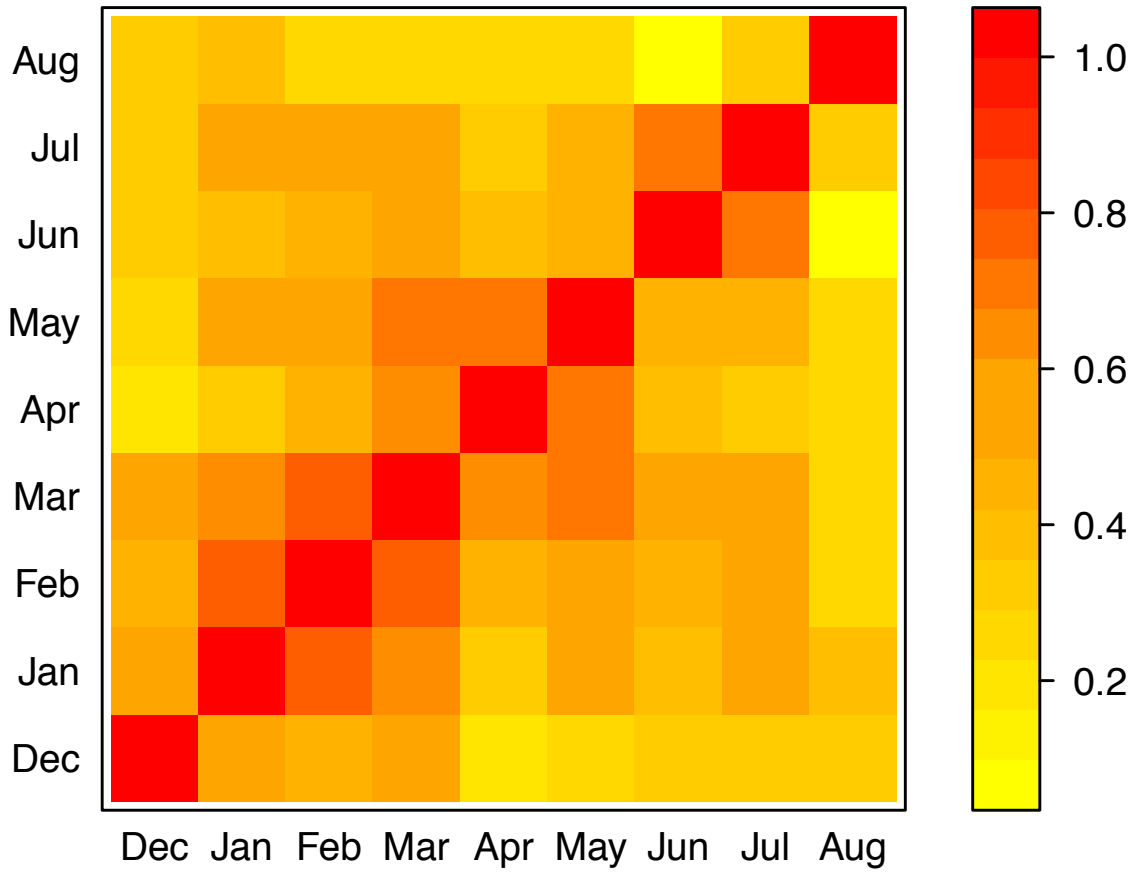

Figure A.3: Stability of party association networks over time. Shading represents Pearson correlation coefficients for pairs of monthly party networks, with yellow representing low correlations and red representing high correlations. All pairwise month combinations were significantly correlated as compared to randomized networks, indicating that the social structure was not random.

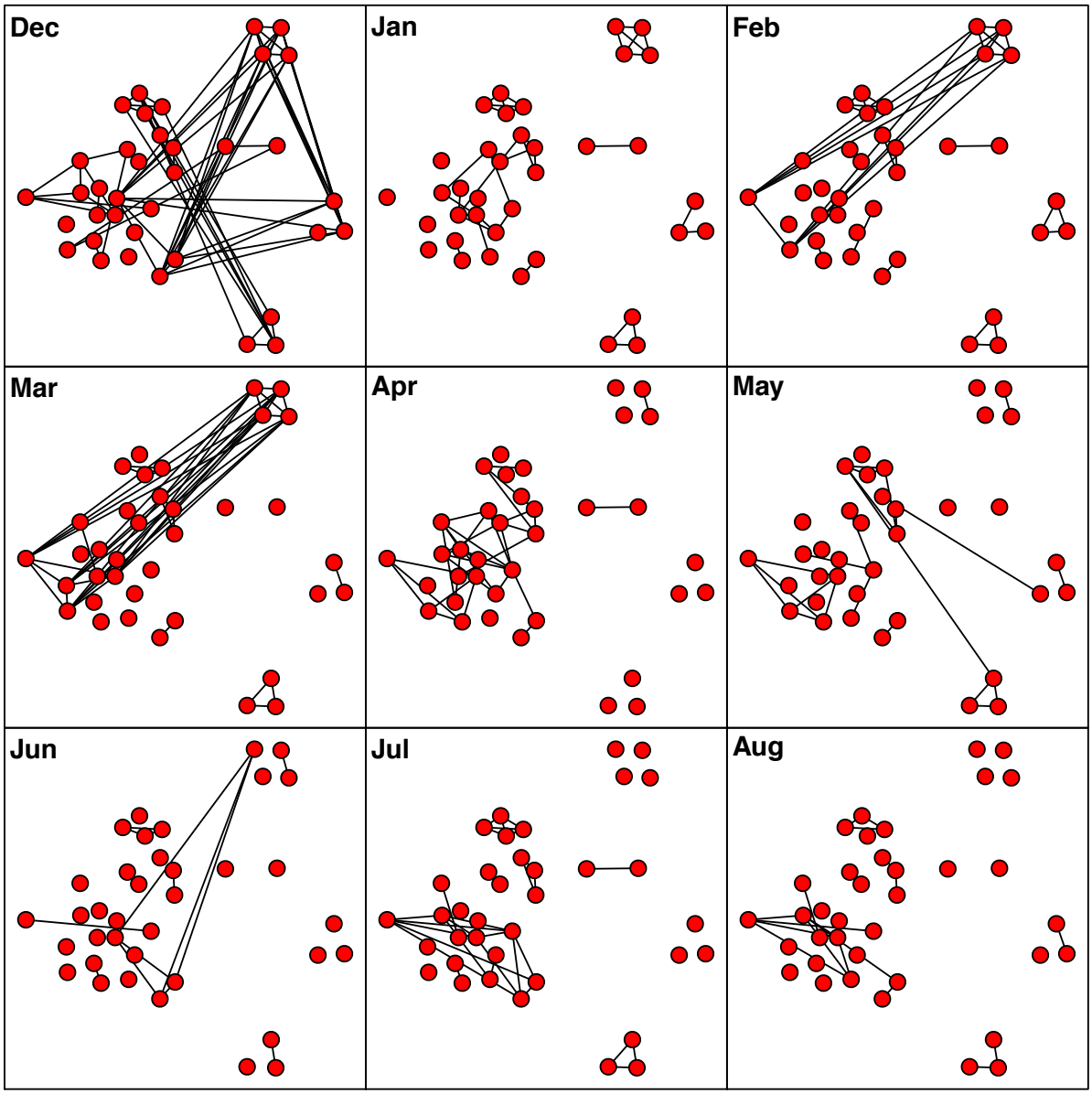

Figure A.4: Monthly 5m association networks. Observed 5m association networks are shown for each of the nine study months. Nodes (circles) represent individual chimpanzees (n = 37) and edges (lines) represent observed associations, where edge thickness corresponds to the pairwise 5m association index (5mAI) between nodes. All networks are displayed with identical layouts and only edges with PAIs > 0.1 are shown. All 37 individuals in the community are displayed as nodes regardless of whether they have a connection. Networks were constructed in R with the igraph package version 0.5.5-4 (Csardi and Nepusz 2006).

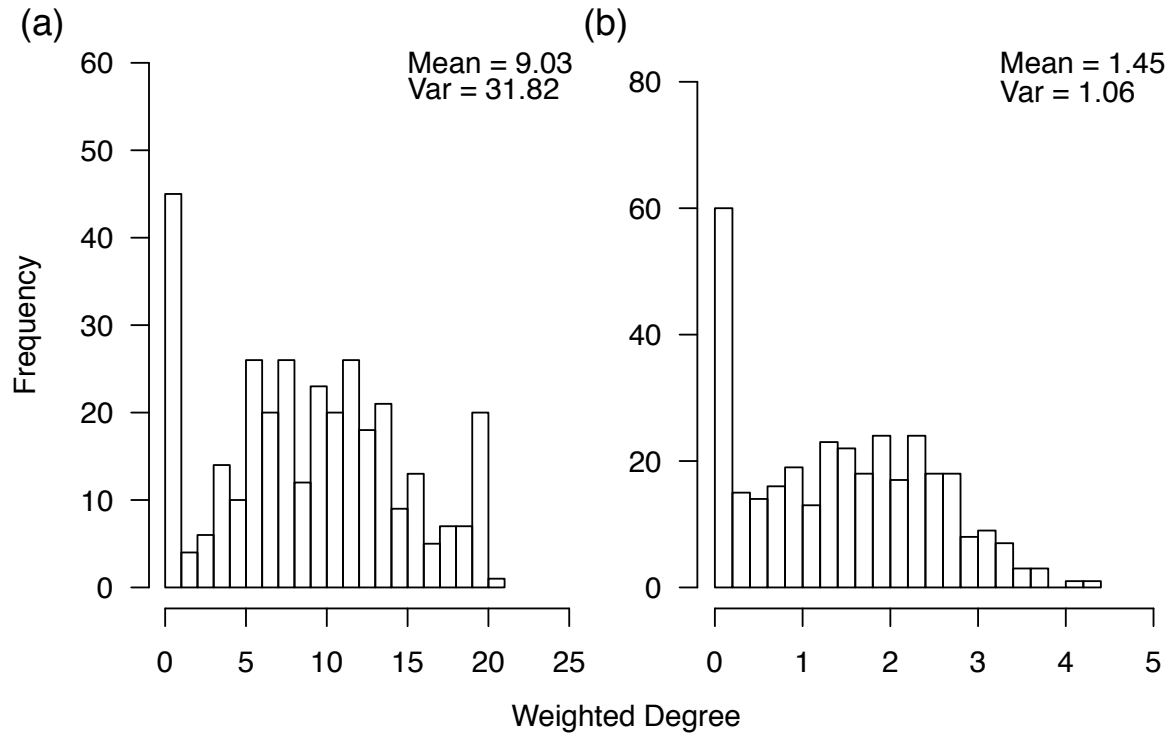

Figure A.5: Overall degree distributions of all individuals ($n = 37$) across all study months ($n = 9$) for a) monthly party association networks and b) monthly 5m association networks. The means and variances are shown for each distribution. While all individuals were not observed every month, we used the party and within-party mixed effects models presented in the main text to predict network degree values for unobserved individuals in a given month. Thus, the degree distributions presented display both observed degree (for observed individuals, where the total sample of observed individuals by month was $n = 294$) and estimated degree (for unobserved individuals, where the total sample of unobserved individuals by month was $n = 39$).

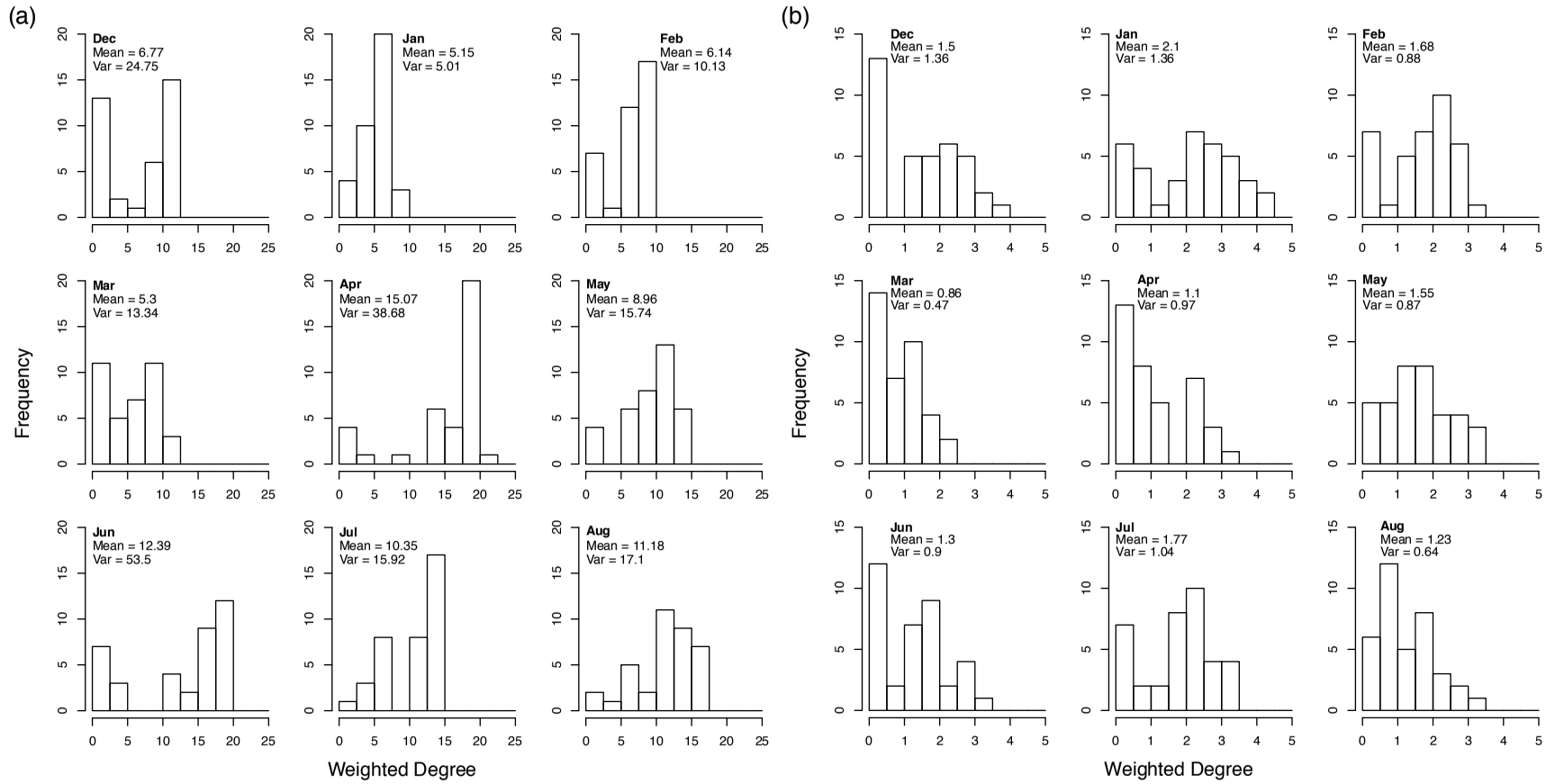

Figure A.6: Degree distributions for all individuals ($n = 37$) broken down by month in a) party association networks and b) 5m association networks. The means and variances in a given month are shown for each degree distribution. While all individuals were not observed every month, we used the party and within-party mixed effects models presented in the main text to estimate degree values for unobserved individuals in a given month. Thus, the degree distributions presented display both observed degree (for observed individuals, where the total sample of observed individuals by month was $n = 294$) and estimated degree (for unobserved individuals, where the total sample of unobserved individuals by month was $n = 39$). For comparison, Figure A.7 presents degree distributions for only observed individuals.

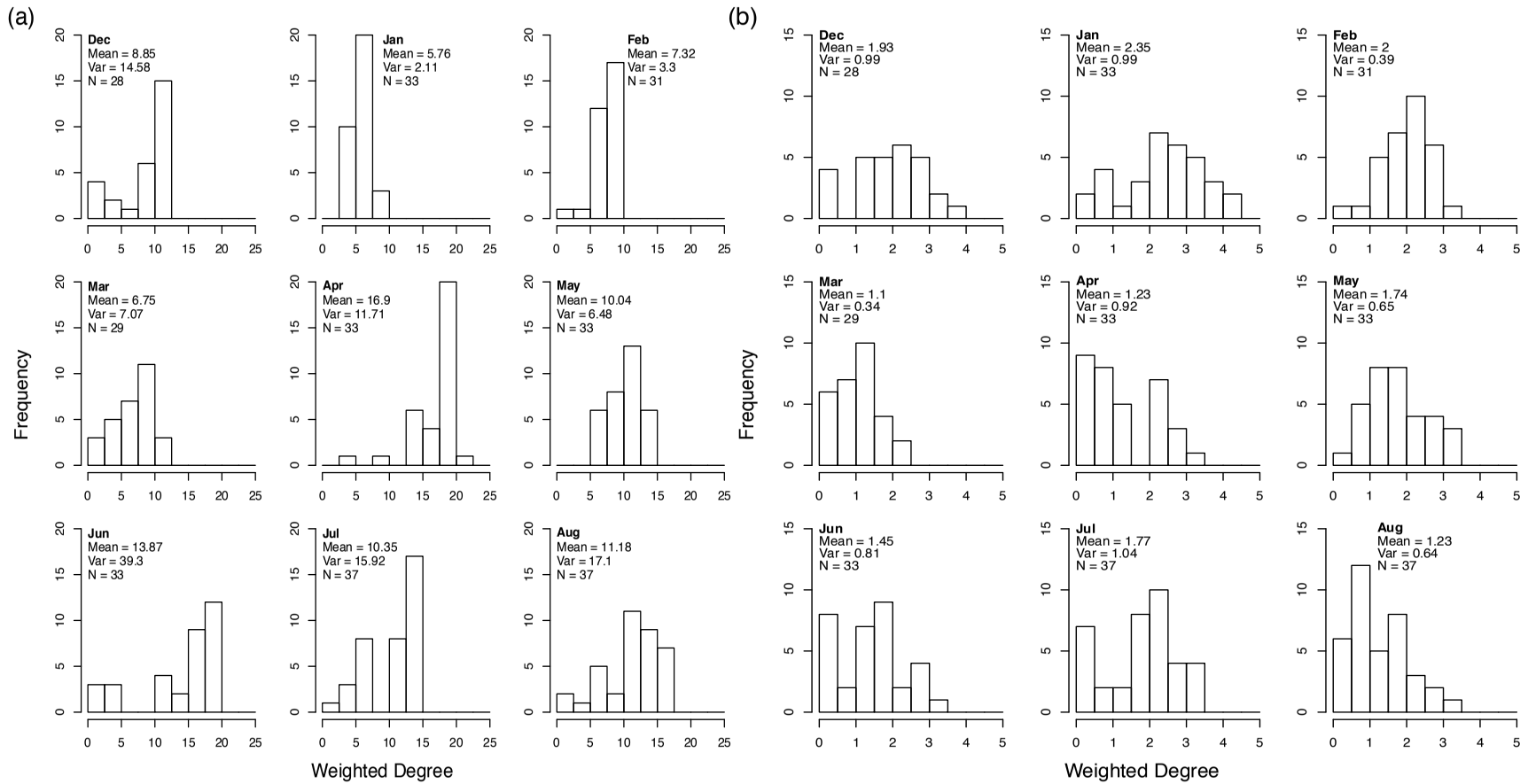

Figure A.7: Degree distributions for observed individuals broken down by month in a) party association networks and b) 5m association networks. The means, variances, and number of individuals observed (out of the total $n = 37$) in a given month are shown for each degree distribution. Unlike Figures A.5 and A.6 (where degree was estimated for unobserved individuals using the mixed effects models presented in the main text), here the means and variances were calculated using the degree values only for individuals that were observed in a given month. Similarly, only observed individuals are presented in the histograms.

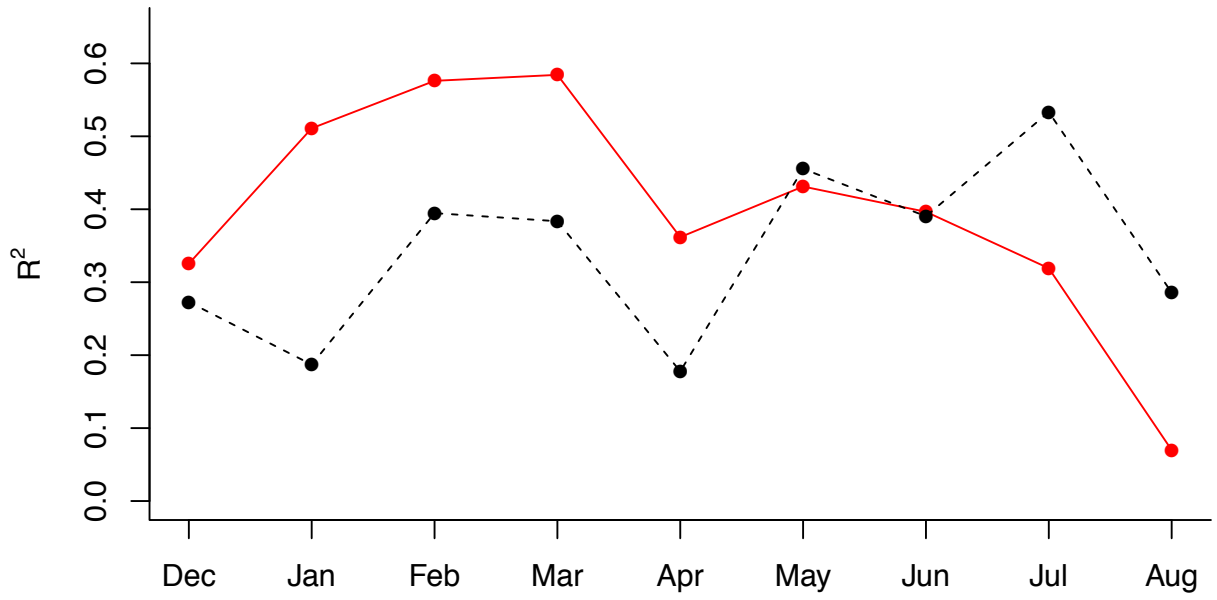

Figure A.8: Goodness of fit for monthly party and within-party association models. R^2 values are shown for monthly PAIs (solid red line) and WPAIs (dashed black line), based on comparisons between logistic mixed-effect model estimates and observed values.

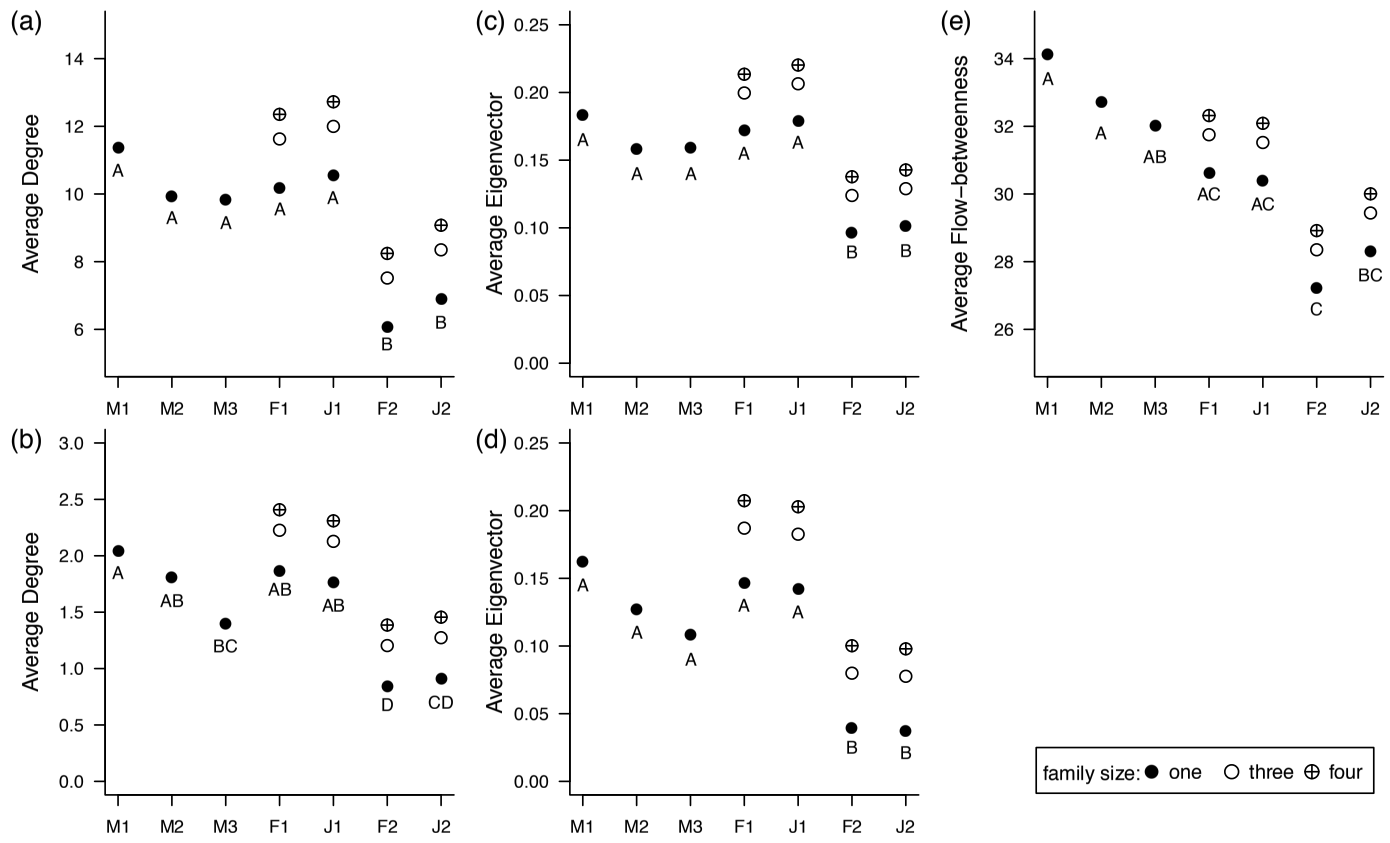

Figure A.9: Estimated effects of rank and family size on average (a-b) degree, (c-d) eigenvector, and (e) flow-betweenness centrality. Top (a, c, e) and bottom (b, d) rows correspond to party and 5m-networks, respectively. There was a significant positive relationship between an individual's family size and degree centrality. Black, white, and cross-symbol circles represent model estimates for individuals with family sizes of one, three, and four members respectively. By definition of family unit, adult male ranks are only presented with a family size of one. Letters on plots show which rank categories were significantly different, after controlling for family size and estrus. Tests not shown for 5m flow-betweenness due to poor model fit ($R^2 = 0.032$, $\mathrm{F}_1 = 0.568$, $P = 0.918$) and no significant parameters (Table 2.2).

Appendix B

SUPPORTING INFORMATION FOR CHAPTER 3

B.1 Supplementary Text

B.1.1: Additional Methods

Quantifying contact networks

To quantify party association indices (PAIs) used to weight party network edges, we determined the number of scans in which chimpanzees A and B were observed in the same party relative to the total number of scans in which either A or B was observed in any party:

$$PAI_{AB} = \frac{S_{AB}}{S_A + S_B + S_{AB}} \tag{B.1}$$

where S_{AB} represents scans where A and B were observed in the same party, S_A represents scans where A was observed in a party without B, and S_B represents scans where B was observed in a party without A. To quantify 5m association indices (5mAIs) used to weight edges of proximity networks, we calculated the probabilities that individuals A and B would be both within the same party and within 5m of each other:

$$5mAI = PAI_{AB}\left(\frac{S_{AB5}}{S_{AB}}\right) \tag{B.2}$$

where S_{AB5} represents scans where A and B were observed within 5m of each other. Thus, this index, which could range from 0 to 1, represents the overall proportion of time that individuals A and B were within 5m of each other.

A major challenge in quantifying contact networks for wildlife is that it is often difficult to observe all study subjects within a given time frame. In our monthly networks, 1.72% of the monthly pairwise interactions were undefined because neither individual A or B was observed within the given month, making it impossible to directly quantify the amount of time the two individuals spent together. To circumvent this issue, we used a Bayesian logistic mixed

effects model (with pairwise predictor variables of age, sex, relatedness, difference in rank, difference in family size, and number of females in estrus) to predict the missing pairwise association indices. The model details are described in Rushmore et al. (In review).

Simulating infectious disease transmission on observed networks

The following three equations demonstrate how we calculated $\beta\tau$ values that were used in pathogen transmission simulations. First, for each individual in each monthly network, we set $R_{0(i,m)}$ for an individual (i) in a given month (m) to the sum of the transmission probabilities between individuals i and j in the network:

$$R_{0(i,\,m)} = \sum_{i \neq j} 1 - e^{-c_{ij}\beta\tau} \tag{B.3}$$

where c_{ij} refers to the association index between i and j in a given monthly network; $\beta\tau$ is not yet known and will be solved for in the equations below. We then set $R_{0(m)}$ for a monthly network to the mean of $R_{0(i,m)}$ for all 37 individuals in that monthly network:

$$R_{0(m)} = \frac{1}{37} \sum_{i} R_{0(i,\,m)} \tag{B.4}$$

The average R_0 ($\overline{R_0}$) across networks was then equivalent to the mean of $R_{0(m)}$ for all 9 monthly networks:

$$\overline{R_0} = \frac{1}{9} \sum_{m} R_{0(m)} = \frac{1}{9} \sum_{m} \frac{1}{37} \sum_{i} \sum_{i \neq j} 1 - e^{-c_{ij}\beta\tau} \tag{B.5}$$

Using a range of biologically relevant estimates for $\overline{R_0}$ (see Methods), we fixed $\overline{R_0}$ and used a root solver in R to calculate corresponding $\beta\tau$ values.

Our $\overline{R_0}$ calculations strictly correspond to the reproductive rate definition typically used in epidemiology literature, where R_0 is the average number of secondary infections caused

by one primary infection in a completely nave population (Anderson et al. 1986). This R_0 definition (hereafter referred to as PR_0 for primary infection R_0) has been used by others to model pathogen transmission on contact networks (Davis et al. 2008; Hamede et al. 2011). However, several other studies use an alternate definition for R_0 in reference to network epidemiology, in which R_0 is the number of secondary infections caused by a randomly selected infected node (i.e., not the index case: Meyers 2007; Newman 2002). We will henceforth refer to this second definition as SR_0 (for secondary infection R_0).

PR_0 depends only on mean degree of the population and edge-specific transmissibilities (T_{ij}) of the pathogen, whereas SR_0 also depends on the variance of the degree distribution and clustering. Hence, using the basis of the PR_0 definition (which was then averaged across months to create $\overline{R_0}$ as described above) allowed us to assess the impact of network structure (month) on outbreak size for a pathogen characterized by a particular $\overline{R_0}$, without the circular issue of $\overline{R_0}$ also being dependent on inherent aspects of network structure (e.g., degree distribution and clustering). Furthermore, using the definition of PR_0 allowed us to easily calculate $\overline{R_0}$ for a given $\beta\tau$ (pathogen infectiousness and duration of infection), by averaging the expected number of secondary cases for each of the possible 37 index cases across the nine monthly networks. Using the definition of SR_0 to calculate $\overline{R_0}$ would require substantially more complicated simulations (i.e., calculating the expected number of secondary cases that result from a randomly selected secondary case for each of the 37 index cases across all nine months). Also, SR_0 may not be appropriate for simulating pathogen transmission on small networks, as basic reproductive rate estimates based on the number of secondary cases could be biased due to the network already being saturated. Lastly, we note that because PR_0 refers to the reproductive rate for an index case, which on average will have a lower mean degree than a secondary case, our $\overline{R_0}$ calculations are expected to be slightly lower than SR_0 calculations.

Permutation tests

The permutation-based regression test (Hanneman and Riddle 2005) uses node-level parameters (where each row in the dataset represents a node and columns provide attribute data for each node). The test first calculates the regression slope coefficients of the observed dataset; then, over 30,000 permutations, the algorithm randomly shuffles values of the dependent variable while leaving the values of the independent variables in place. Regression coefficients are recalculated after each permutation, and the P-value for each parameter is calculated by determining the proportion of permutations that yielded values as or more extreme than the original regression for the observed dataset. This test controls for the interdependencies of network nodes. Because we observed a non-linear relationship between mean outbreak size and $\overline{R_0}$, we ran a separate model for each of the four $\overline{R_0}$ values.

Calculating centrality measures

We calculated weighted centrality metrics (degree, eigenvector, betweenness) for each individual in each month using UCINET (Borgatti et al. 2002).

B.1.2: Information on Kibale Forest and the Kanyawara Chimpanzee Community

Struhsaker (1997) provides an overview of Kibale National Park, including forest ecology. The Kanywara chimpanzee community, which occupies roughly 37.8 km^2 of Kibale (Wilson et al. 2001), was partially habituated to human presence by M. Ghiglieri during 1979-1980 and G. Isabirye-Basuta during 1983-1985. The Kanyawara community was fully habituated to human observers by 1990 (Wrangham et al. 1992). R. Wrangham founded the Kibale Chimpanzee Project (KCP) in 1987, and along with colleagues, has continuously collected data on chimpanzee life history, behavioral development, and social relationships (among

other topics). Funding for KCP is provided by the U.S. National Science Foundation (awards 9807448 and 0416125), the U.S. National Institutes of Health, National Geographic Society, L.S.B. Leakey Foundation, and the Wenner-Gren Foundation.

To collect behavioral data for this paper, J. Rushmore worked alongside KCP researchers and field assistants. At the time of this study, the community was comprised of 48 chimpanzees with 12 adult males (aged > 14), 14 adult females (aged > 13), 9 immature males and 6 immature females (aged between 5-14 and 5-13 respectively; referred to throughout the main text as juveniles), and 7 dependent offspring (aged ≤ 4).

B.2 Supplementary Tables

Table B.1: Chimpanzee trait data for study subjects ($n = 37$). This table shows the sex, age class, family size, and trait-based group for all chimpanzees included in the study.

Chimpanzee ID	Sex[1]	Age Class[2]	Family Size	Trait-based Group[3]
AJ	M	A	1	HM
AL	F	A	3	CR-L
AT	M	J	3	CR-L
AZ	M	J	3	CR-L
BB	M	A	1	HM
BL	F	A	3	ER
BO	M	J	3	ER
BU	F	J	3	ER
ES	M	A	2[4]	MM
EU	F	J	2[4]	CR-S
KK	M	A	1	HM
LK	M	A	1	HM
LR	F	A	1	CR-S
ML	F	A	1	ER
MS	M	A	1	HM
MU	F	A	2	ER
MX	M	J	2	ER
NP	F	J	1	CR-S
OG	M	J	4	CR-L
OM	F	J	4	CR-L
OT	F	J	4	CR-L
OU	F	A	4	CR-L
PB	M	A	1	LM
PG	M	A	1	MM
QT	F	A	1	CR-S
RD	F	A	1	ER
ST	M	A	1	MM
TG	F	A	4	CR-L
TJ	M	A	4	LM
TS	F	J	4	CR-L
TT	M	J	4	CR-L
TU	M	A	1	MM
UM	F	A	2	ER
UN	M	J	2	ER
WA	F	A	1	ER
WL	F	A	1	CR-S
YB	M	A	1	LM

[1]Sex: M=male, F=female

[2]Age Class: A=adult, J=juvenile (as defined in the main text)

[3]Trait-based group: HM, MM, and LM refer to high-, medium-, and low-ranking adult males, respectively; CR-L refers to core-ranging adult females and juveniles with families larger than 2 members; CR-S refers to core-ranging adult females and juveniles with families smaller than 3 members; ER refers to edge-ranging adult females and juveniles

[4]Even though their mother was deceased, ES and EU (a brother-sister pair) were considered a family unit, as ES and EU spent more than 65% of their time together

Table B.2: Comparison of minimum coverage requirements across vaccination strategies. For each vaccination strategy, the coverage threshold is provided as a percentage of the community, with the number of individuals vaccinated in parentheses, for A) the mean outbreak size to affect $< 10\%$ of the community (Minimum Coverage Threshold), and B) an outbreak to affect $< 10\%$ of the community in at least 95% of the simulations (Conservative Coverage Threshold). The table shows results for trait-based simulations using a single adult male category (M). Results for trait-based vaccinations represent simulations with a single adult male category. Results were identical for simulations using this category M or three adult male categories (HM, MM, LM; see Chapter 3 Results), except for a couple instances (*) in which simulations using HM, MM, and LM categories required vaccinating one less individual.

A. Minimum Coverage Threshold:				
Vaccination strategy	$\overline{R_0} = 0.7$	$\overline{R_0} = 1.5$	$\overline{R_0} = 3.0$	$\overline{R_0} = 10.0$
Centrality-based	0% (0)	2.70% (1)	18.92% (7)	32.43% (12)
Trait-based	0% (0)	2.70% (1)	21.62% (8)*	32.43% (12)
Random	0% (0)	5.41% (2)	24.32% (9)	37.84% (14)
B. Conservative Coverage Threshold:				
Vaccination strategy	$\overline{R_0} = 0.7$	$\overline{R_0} = 1.5$	$\overline{R_0} = 3.0$	$\overline{R_0} = 10.0$
Centrality-based	0% (0)	27.03% (10)	45.95% (17)	62.16% (23)
Trait-based	5.41% (2)	35.14% (13)*	45.95% (17)	64.86% (24)
Random	8.11% (3)	43.24% (16)	56.76% (21)	67.57% (25)

B.3 Supplementary Figures

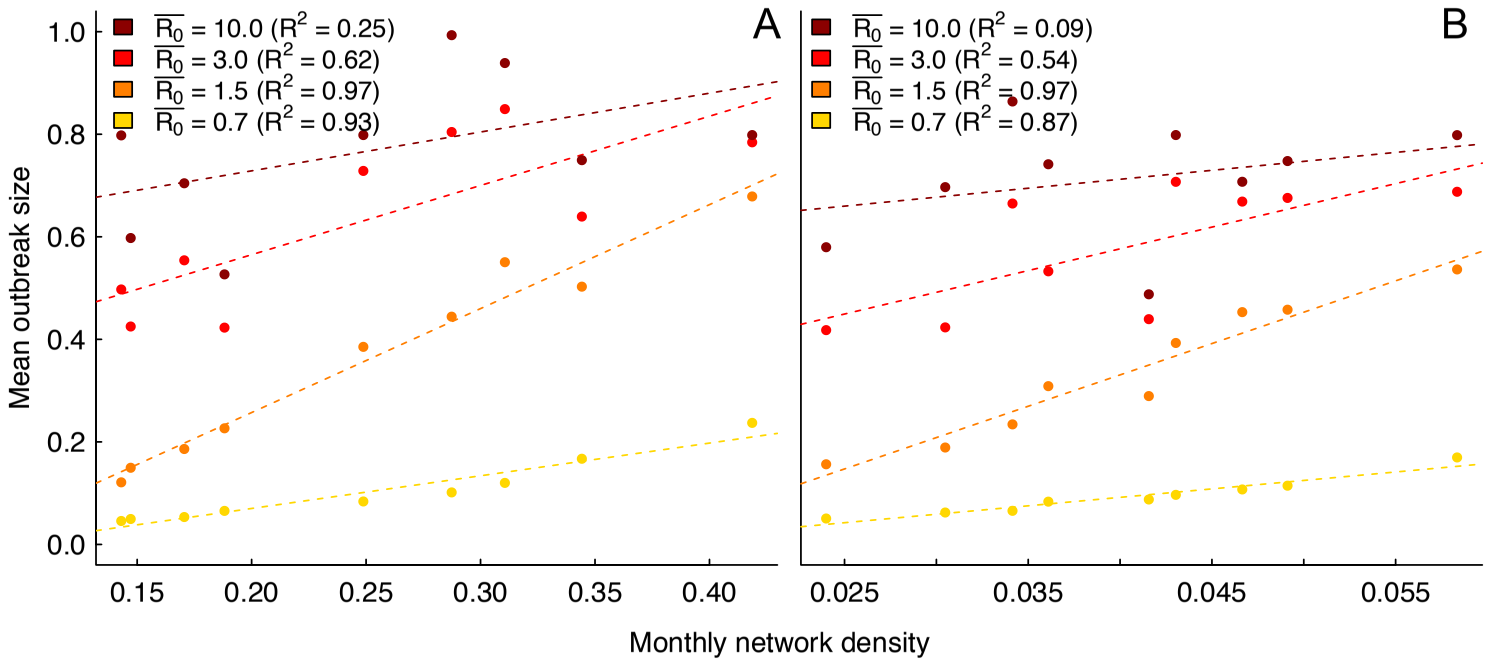

Figure B.1: Relationship between monthly network density and mean outbreak size. Correlations are shown between monthly network density and mean outbreak size (averaged across all index cases) for four different $\overline{R_0}$ values in party networks (A) and proximity networks (B).

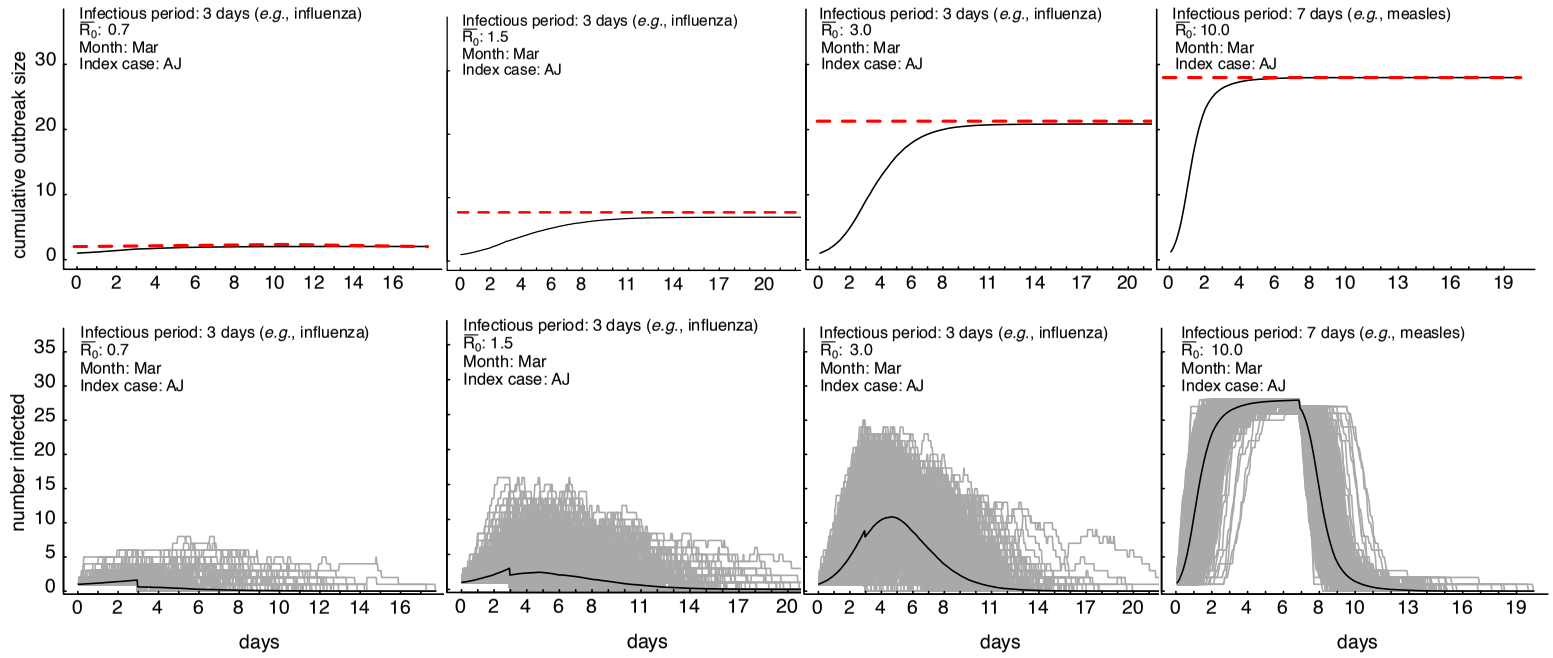

Figure B.2: Comparing bond percolation and temporal chain-binomial model outcomes for pathogen transmission. The top row shows cumulative outbreak size over time for a range of $\overline{R_0}$ values (by panel) using a temporal chain binomial model with a fixed recovery rate (solid black line) and the mean final outbreak size as determined by bond percolation (dotted red line). These panels confirm that chain binomial and bond percolation model outcomes were consistent. The bottom row shows 1000 simulations (grey lines) of the number of infected individuals over time for a range of $\overline{R_0}$ values (by panel) using a temporal chain binomial model. The solid black line shows the average number of individuals infected over time (i.e., averaged across the 1000 simulations).

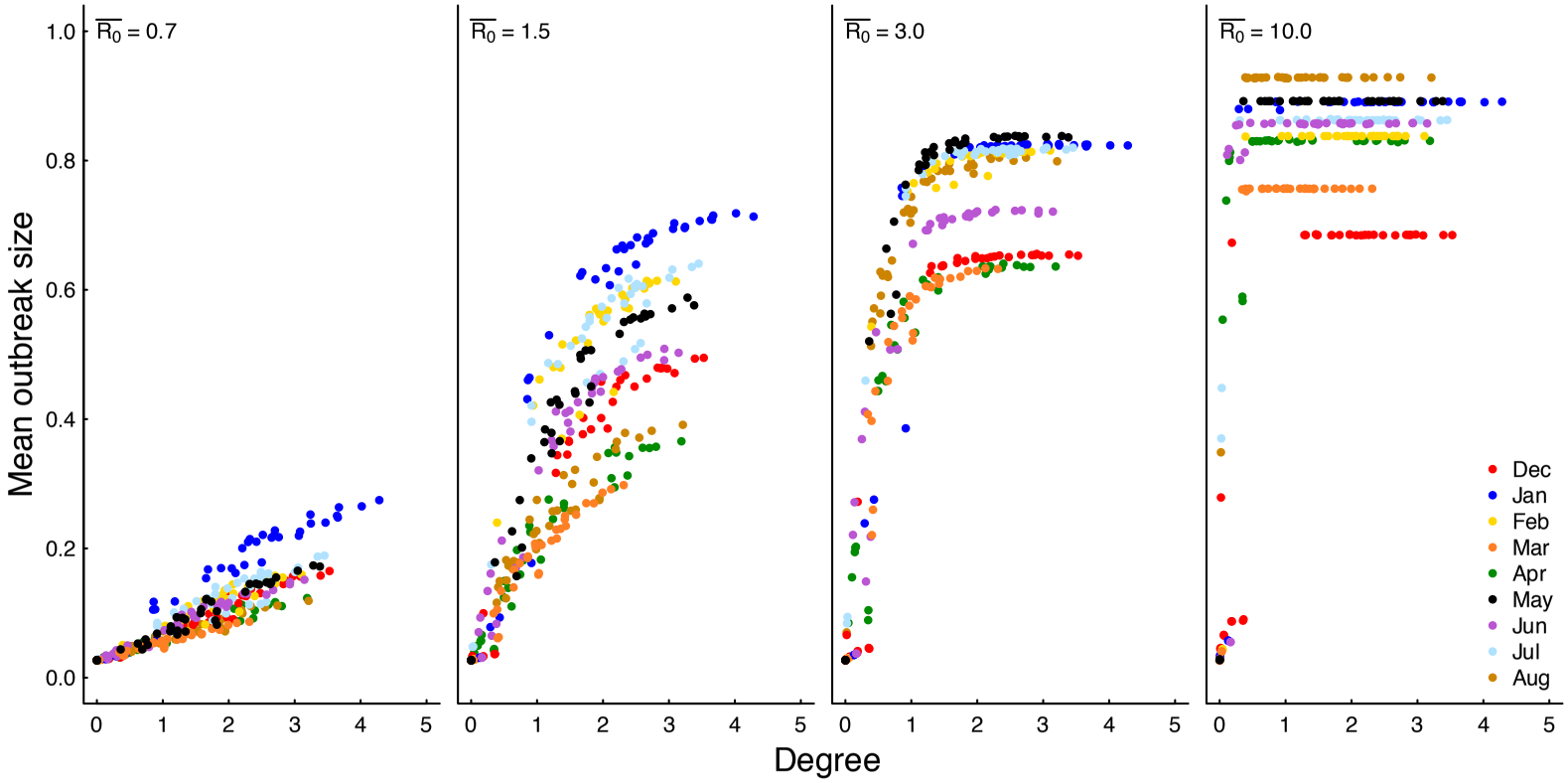

Figure B.3: Mean outbreak size increases with the degree centrality of the index case. Mean outbreak sizes are shown (as proportions of the community) against degree centrality of the index case for proximity networks. Panels show different levels of pathogen infectiousness (left to right: $\overline{R_0} = 0.7$, 1.5, 3.0, 10.0), and data point colors represent different monthly networks. Thus, there are 37 data points (representing each possible index case) for each month within a given value of $\overline{R_0}$. Estrous females were present during Jan ($n = 1$), Apr ($n = 1$), May ($n = 1$), Jun ($n = 1$), Jul ($n = 2$), and Aug ($n = 2$).

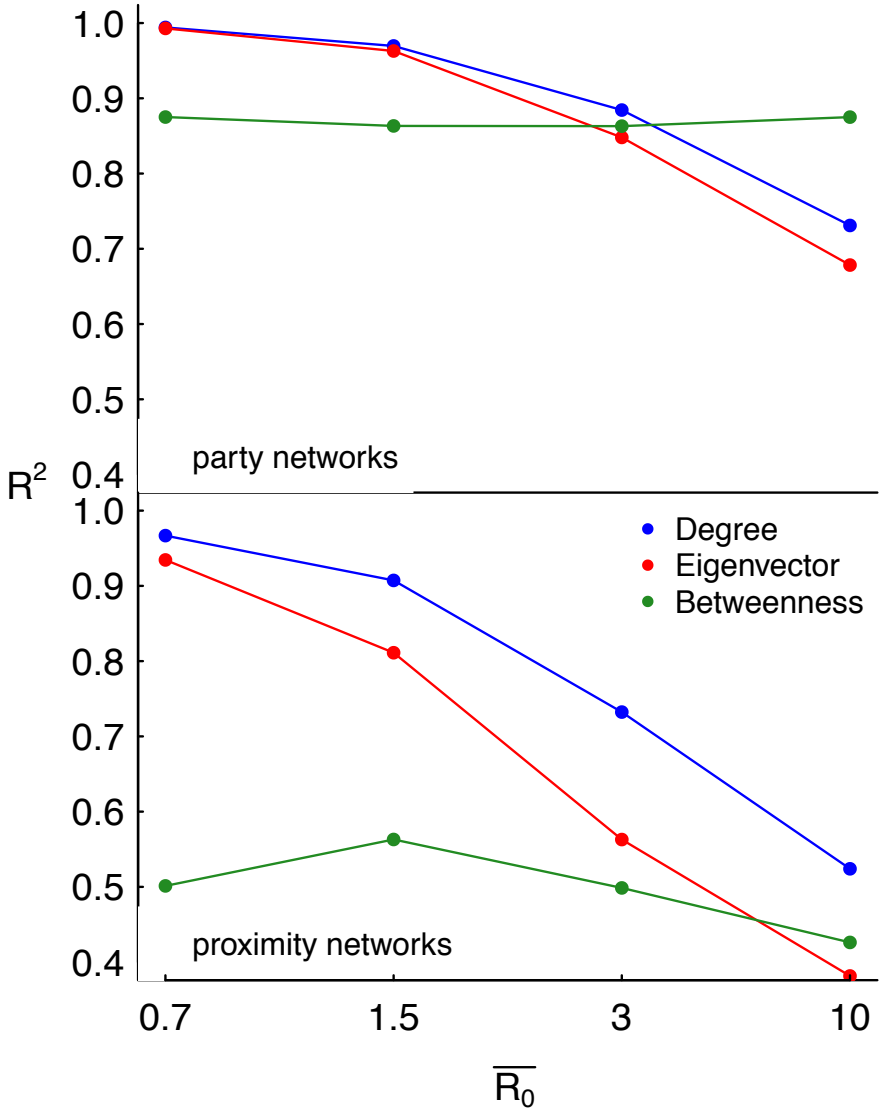

Figure B.4: Correlations of mean outbreak sizes and centrality measures of index cases. Correlations (R^2) for mean outbreak size and three weighted centrality measures of the index case (degree: blue, eigenvector: red, betweenness: green) are shown for varying levels of pathogen infectiousness ($\overline{R_0}$) in party networks (top panel) and proximity networks (bottom panel).

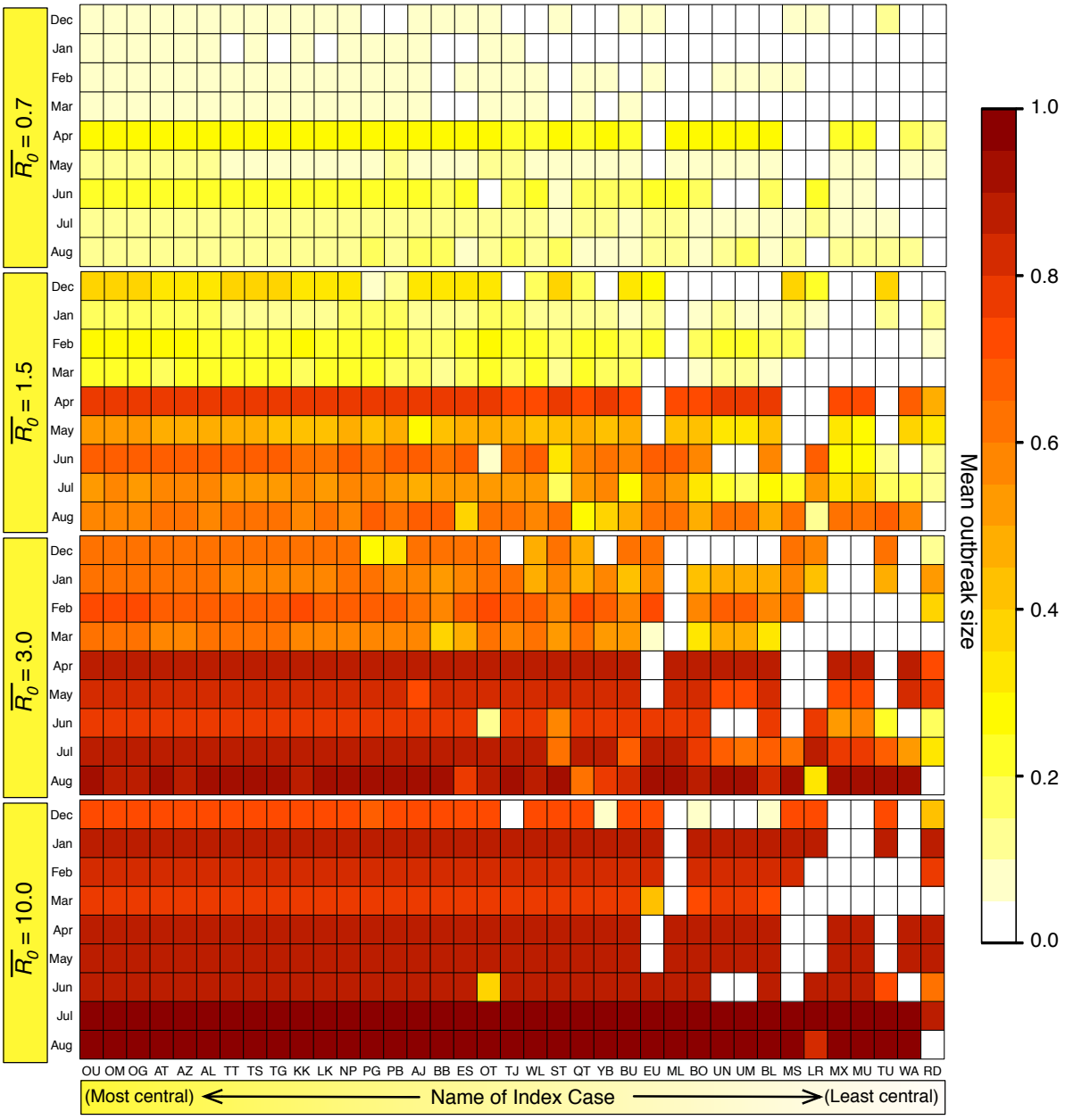

Figure B.5: Mean outbreak size results for pathogen transmission simulations on party-level chimpanzee networks. The color of each cell shows the average proportion of the community ($n = 37$) that was infected across the 1000 replicates per unique combination of parameters The x-axis shows the identities of the index cases, ordered from highest to lowest mean degree centrality (i.e., averaged across months). Estrous females were present during Jan ($n = 1$), Apr ($n = 1$), May ($n = 1$), Jun ($n = 1$), Jul ($n = 2$), and Aug ($n = 2$).

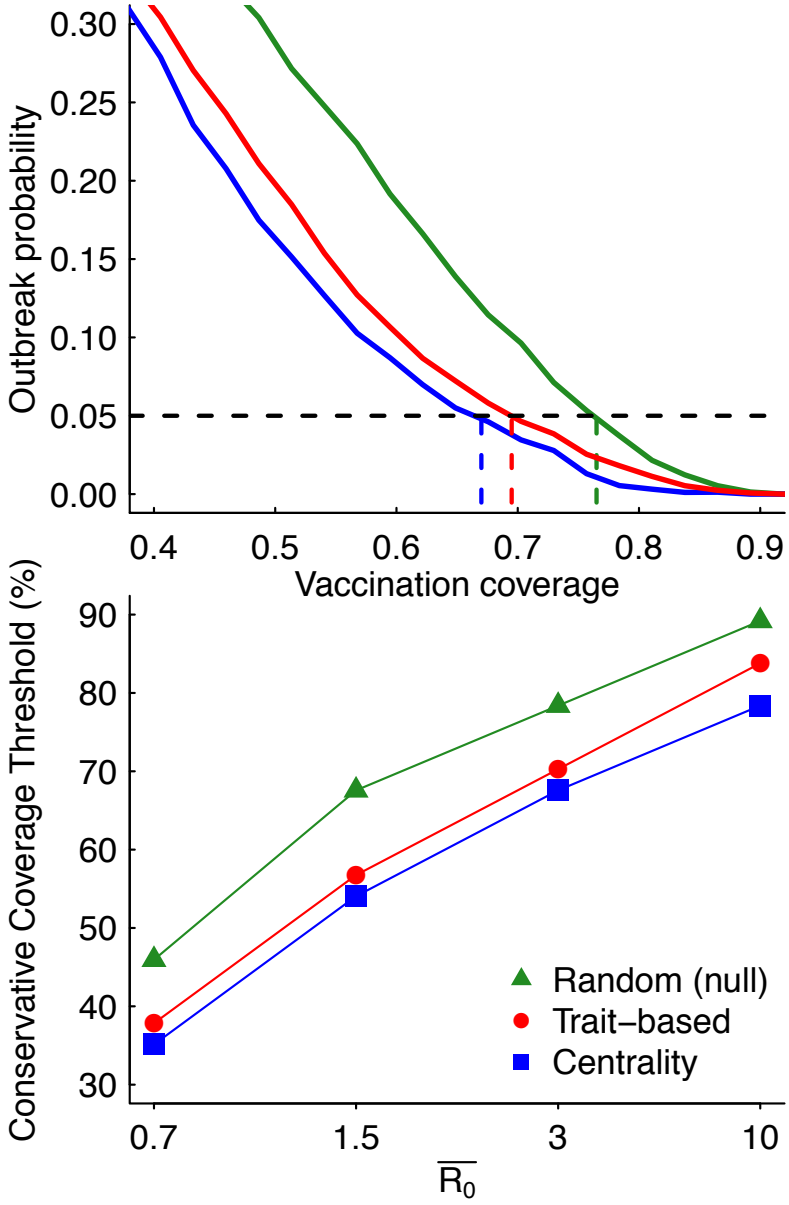

Figure B.6: Evaluation of vaccination strategies on party networks by the Conservative Coverage Threshold. The top panel shows the outbreak probability (the proportion of simulations resulting in an outbreak greater than 10% of the community) for centrality-based vaccinations (blue), trait-based vaccinations (red), and random vaccinations (green) at varying levels of coverage (shown as a proportion of the community) when $\overline{R_0} = 3.0$. The black dotted line marks the Conservative Coverage Threshold, at which no more than 5% of the simulations result in outbreaks. The bottom panel shows this Conservative Coverage Threshold for each vaccination strategy and $\overline{R_0}$ combination.

Appendix C

SUPPORTING INFORMATION FOR CHAPTER 4

C.1 Supplementary Tables

Table C.1: Wild Kanyawara chimpanzee subjects from Kibale National Park, Uganda. Urine samples were screened for trichomonads and *Chlamydia* spp. Fecal samples were screened for trichomonads. The table shows results for trichomonads; all samples were negative for *Chlamydia* spp. Table continued onto the next page.

ID	Sex	Age Class at Sample Collection[a]	Total Urine Samples	Positive Urine Samples	Fecal Samples Collected	Positive Fecal Samples
AuntieRose	Female	Adult	2	2	0	NA
BadFoot	Male	Adult	1	0	0	NA
Beatle	Male	Immature	2	0	0	NA
Big Brown	Male	Adult	8	5	2	0
Bono	Male	Immature	5	0	0	NA
Bubbles	Female	Adult	2	1	2	2
Bud	Male	Adult	17	8	2	2
Budongo	Female	Immature	4	2	6	6
Edward	Male	Adult	3	1	0	NA
Ekisigi	Female	Adult	2	2	0	NA
Eslom	Male	Adult	7	2	4	2
Euro	Female	Immature	6	5	3	2
Finger	Female	Adult	3	2	0	NA
Goodall	Female	Adult	1	1	0	NA
Harare	Female	Adult	1	0	0	NA
Imoso	Male	Adult	6	2	2	0
Ipassa	Female	Adult	2	0	0	NA
Johnny	Male	Adult	6	2	0	NA
Josta	Female	Adult	3	2	0	NA
Kaana	Female	Adult	1	1	0	NA
Kabarole	Female	Adult	1	1	0	NA
Kakama	Male	Adult	13	5	2	1
Kilimi	Female	Adult	2	1	0	NA
Lanjo	Male	Adult	8	2	3	3
Lia	Female	Adult	11	5	2	1
Light Brown	Male	Adult	1	1	0	NA
Likizo	Male	Immature	4	1	1	0
Lope	Female	Adult	1	1	0	NA
Makoku	Male	Adult	11	2	2	2
Mandela	Male	Immature	4	1	0	NA
Max	Male	Immature	2	0	0	NA

Continued onto the next page…

[a]Infants (≤ 4 y), Immatures (males: 5 – 14 y, females: 5 – 13 y), Adults (males: > 14 y, females > 13 y)
[b] NA = Not applicable: the given sample type was not available for the given individual

Table C.1 (continued)

ID	Sex	Age Class at Sample Collection[a]	Total Urine Samples	Positive Urine Samples	Fecal Samples Collected	Positive Fecal Samples
Continued from the previous page…						
Michelle	Female	Adult	2	1	3	2
Mususu	Female	Adult	6	1	2	1
Nectar	Female	Immature	2	0	0	NA
Ngamba	Female	Immature	1	1	0	NA
Nile	Female	Adult	2	0	0	NA
Nyenka	Female	Adult	3	3	0	NA
Omusisa	Female	Immature	7	3	1	1
Outamba	Female	Adult	14	7	2	1
Quinto	Female	Adult	3	0	1	1
Rafiki	Male	Immature	1	0	0	NA
Rosa	Female	Adult	10	6	0	NA
Rwanda	Female	Adult	1	0	1	1
Sanyu	Female	Immature	1	1	0	NA
Slim	Male	Adult	1	0	0	NA
Special	Female	Immature	5	3	2	2
Stocky	Male	Adult	1	0	0	NA
Stout	Male	Adult	5	3	4	2
Stump	Female	Adult	2	1	0	NA
Tacugama	Male	Immature	8	2	3	3
Teddy	Female	Infant	2	1	0	NA
Tenkere	Female	Immature	5	0	2	1
Tofu	Male	Adult	4	2	3	1
Tongo	Female	Adult	10	4	1	1
Tsunami	Female	Immature	4	1	0	NA
Tuber	Male	Immature	2	1	1	1
Tuke	Male	Immature	8	2	2	1
Twig	Male	Adult	22	7	0	NA
Umbrella	Female	Adult	3	1	5	3
Wangari	Female	Adult	2	1	2	1
Wilma	Female	Adult	10	2	2	2
Yogi	Male	Adult	7	4	1	1

[a]Infants (≤ 4 y), Immatures (males: 5 – 14 y, females: 5 – 13 y), Adults (males: > 14 y, females > 13 y)
[b] NA = Not applicable: the given sample type was not available for the given individual

Table C.2: Wild Sonso chimpanzee subjects from Budongo Forest, Uganda. Urine samples were screened for trichomonads and *Chlamydia* spp. The table shows results for trichomonads; all samples were negative for *Chlamydia* spp.

ID	Sex	Age Class at Urine	Number of Urine Samples	Number of Positive Urine Samples
Bwoba	Male	Adult	2	0
Bahati	Female	Immature	3	1
Black	Male	Adult	1	1
Banura	Female	Adult	1	0
Bob	Male	Adult	2	0
Beti	Female	Immature	1	0
Clea	Female	Adult	1	0
Duane	Male	Adult	2	0
Emma	Female	Immature	3	3
Fred	Male	Immature	1	0
Gashom	Male	Adult	2	1
Gonza	Female	Immature	3	0
Harriet	Female	Adult	3	1
Hawa	Male	Immature	3	0
Juliet	Female	Adult	2	1
Jambo	Male	Adult	3	2
Janie	Female	Adult	1	1
Janet	Female	Infant	2	0
Katia	Female	Infant	3	0
Keti	Female	Immature	1	1
Kigere	Female	Adult	1	0
Kalema	Female	Adult	3	1
Kana	Female	Immature	1	0
Kato	Male	Immature	3	1
Kutu	Female	Adult	1	0
Kwera	Female	Adult	1	0
Kewaya	Female	Adult	3	3
Kwezi	Male	Immature	4	2
Maani	Male	Infant	2	0
Mukwano	Female	Adult	1	0
Melissa	Female	Adult	1	1
Mark	Male	Immature	1	0
Musa	Male	Adult	3	0
Nambi	Female	Adult	1	0
Nkojo	Male	Adult	3	0
Nick	Male	Adult	2	0
Nora	Female	Immature	3	1
Rachel	Female	Immature	1	0
Ruhara	Female	Adult	1	0
Sabrina	Female	Adult	3	1
Shida	Female	Immature	3	0
Squibbs	Male	Adult	1	0
Tinka	Male	Adult	2	0
Wilma	Female	Adult	3	2
Zana	Female	Adult	4	0
Zefa	Male	Adult	3	0
Zig	Male	Immature	1	0
Zalu	Male	Immature	1	0
Zimba	Female	Adult	2	1

*Infants (≤ 4 y), Immatures (males: 5 – 14 y, females: 5 – 13 y)
Adults (males: > 14 y, females > 13 y)

Table C.3: Eastern gorilla subjects. Sera samples were screened for *Treponema pallidum*; all samples were negative.

ID	Subspecies	Sex	Date of birth*	Positive samples
Amahoro	*G. b. beringei*	Male	3/20/02	0
Bikenge	*G. b. beringei*	Male	2/3/01	0
Binyindo	*G. b. beringei*	Female	Adult	0
Bukima	*G. b. beringei*	Female	7/3/94	0
Bukumu	*G. b. beringei*	Female	Adult	0
Dufantanye	*G. b. beringei*	Female	10/24/89	0
Dunia	*G. b. graueri*	Female	3/7/06	0
Gukunda	*G. b. beringei*	Female	1/1/73	0
Icyi	*G. b. beringei*	Female	6/13/04	0
Icyzere	*G. b. beringei*	Female	1/1/97	0
Inkumbuza	*G. b. beringei*	Male	Infant	0
Isoni	*G. b. beringei*	Female	Adult	0
Itebero	*G. b. graueri*	Male	11/4/03	0
Joli Ami	*G. b. beringei*	Male	Adult	0
Juma	Unknown	Unknown	Unknown	0
Kaboko	*G. b. beringei*	Male	Infant	0
Kagofero	*G. b. beringei*	Female	Adult	0
Kajoriti	*G. b. beringei*	Male	1/1/94	0
Kibeye	*G. b. beringei*	Female	1/1/88	0
Kidole	*G. b. beringei*	Female	Adult	0
Kubinya	*G. b. beringei*	Female	8/1/81	0
Kureba	*G. b. beringei*	Male	29/03/2003	0
Maisha	*G. b. beringei*	Female	Infant	0
Manzero	*G. b. beringei*	Female	Adult	0
Mararo	*G. b. beringei*	Female	6/1/98	0
Missing Finger	*G. b. beringei*	Female	1/1/85	0
Mpore	*G. b. beringei*	Female	3/20/02	0
Mukecuru	*G. b. beringei*	Female	Adult	0
Mukecuru-Humba	*G. b. beringei*	Female	1/1/69	0
Munyinya	*G. b. beringei*	Male	7/20/2008	0
Mvuyekure	*G. b. beringei*	Female	Infant	0
Mwirakazi	*G. b. beringei*	Female	Unknown	0
Ndunguntse	*G. b. beringei*	Male	Juvenile	0
Ntabwoba	*G. b. graueri*	Male	Infant	0
Okapi	*G. b. beringei*	Male	5/20/02	0
Pasika	*G. b. beringei*	Female	4/1/91	0
Pinga	*G. b. graueri*	Female	9/7/08	0
Puck	*G. b. beringei*	Female	12/15/68	0
Ruvumu	*G. b. beringei*	Female	9/2/95	0
Sebagabo	*G. b. beringei*	Male	Juvenile	0
Serufuli	*G. b. graueri*	Female	3/25/2007	0
Tumaini	*G. b. graueri*	Female	1/19/2005	0
Tuyishimi	*G. b. beringei*	Female	3/21/89	0
Umrava	*G. b. beringei*	Male	1/4/86	0
Umwe	*G. b. beringei*	Female	3/13/00	0
Walanza	*G. b. beringei*	Female	1/1/69	0

* Age class is denoted when date of birth is not known

Table C.4: Oligonucloetide primers used in *Trichomonas* spp. and *Chlamydia* spp. PCR.

Primer ID	Sense	PCR Type[1]	Sequence (5' – 3')
TFR1	Forward	*Trichomonas* primary[1]	TGCTTCAGTTCAGCGGGTCTTCC
TFR2	Reverse	*Trichomonas* primary[1]	CGGTAGGTGAACCTGCCGTTGG
K1-5.8S-100	Forward	*Trichomonas* secondary[2]	GTCTTGGCTCCTCACACGATG
K1-28S-338	Reverse	*Trichomonas* secondary[2]	CTTCAGTTCAGCGGGTCTTCCT
CG-Omp-1-F	Forward	*Chlamydia* primary[3]	AGGTAAGWATGAAAAAACTCTTGAAA
CG-Omp-424-R	Reverse	*Chlamydia* primary[3]	CAGAAWAYATCAAARCGATCCCA
CG-Omp-78-Fi	Forward	*Chlamydia* secondary[3]	CCTGTRGGGAAYCCWGCTGAACCAAG
CG-Omp-219-Ri	Reverse	*Chlamydia* secondary[3]	CGAAAACAWARTCTCCGTAG

Cycling parameters:

[1] 94°C for 2min, 40 cycles of (94°C for 30 sec, 66°C for 30 sec, 72°C for 1 min), 6 min at 72°C

[2] 94°C for 2min, 40 cycles of (94°C for 1 min, 50°C for 1 min, 72°C for 1 min), 10 min at 72°C

[3] 94°C for 2min, 40 cycles of (94°C for 1 min, 50°C for 1 min, 72°C for 1 min), 10 min at 72°C

Table C.5: Model estimates for predictors of urine sample *Trichomonas* spp. infection status (full table).

Predictor	**Estimate**	**SE**	***P***
Intercept	0.57	0.57	0.315
Sex (male)[1]	-0.49	0.23	**0.037**
Age	0.02	0.01	0.112
Season (wet)[2]	-0.62	0.25	**0.014**
Year[3]			
1999	1.69	1.19	0.158
2000	-0.09	1.04	0.934
2001	-0.71	0.76	0.352
2002	-0.40	0.73	0.587
2003	-1.89	0.67	**0.005**
2004	-0.94	0.63	0.136
2005	-0.35	0.67	0.606
2006	-1.87	0.70	**0.008**
2007	-1.35	0.62	**0.029**
2008	-16.19	1373.00	0.991
2009	-0.83	0.57	0.142
2010	-1.08	0.54	**0.045**

[1]Baseline sex is female
[2]Baseline season is dry
[3]Baseline year is 1998

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