

Predicting disease dynamics in African lion populations

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Dedication

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Abstract

In 1994, one-third of Serengeti lions died from canine distemper virus (CDV). I estimated the epidemiological network structure of the Serengeti lion population using long-term data. I found that the lion population is a mix of local pride-to-pride contacts (driven by territory adjacencies) and transient nomad-to-pride contacts (driven by gamma variance process). When canine distemper virus (CDV) was introduced into the network, I found that although nomads are numerous, travel long distances, and are likely candidates to be considered “superconnectors” (connecting distant parts of a network), their impacts on CDV disease dynamics were surprisingly low.

Analysis of the data-driven, Levins-type network model demonstrates that the epidemic probably was not propagated solely by within-species transmission but rather involved multiple introductions from other carnivore species, such as jackals and hyenas. The social network model further suggests that the epidemiological observations from the 2000 km² Serengeti study area may not have reflected the larger-scale dynamics because the sample was (1) located at the periphery of the pride-pride contact network and (2) confined to a small region relative to the scale of the ecosystem.

If lions could not produce the observed CDV outbreak, and other wild carnivores were repeatedly involved in transmission to the lion population, could a multi-host spatial model account for the patchy pattern of CDV spread seen in lions in 1994? A stochastic susceptible-infected-recovered model was constructed which allowed transmission between a highly territorial species, like lions, and 1-2 more gregarious hosts, such as hyenas and jackals. When other gregarious species were coupled with lions with low interspecific contact rates, the erratic patterns of CDV spatial spread were similar to those seen in lions in 1994.

The results of both the network and the multi-host models suggest that lions are a non-maintenance population for canine distemper virus, and more broadly address issues of spatial disease ecology and multi-host pathogens in complex ecosystems.

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CHAPTER 1

Ecology of infectious diseases in Serengeti lions*

Chapter Introduction

Diseases that affect lions (*Panthera leo*) are often connected with much larger ecosystem processes. Pathogens often infect more than one host species; these multi-host pathogens (e.g. rabies, canine distemper virus) link lions to domestic animals and also to populations of endangered wildlife (e.g. African wild dogs) (Cleaveland *et al.* 2002). Second, each host species is commonly infected by more than one pathogen, which can change expected disease transmission rates and virulence (Graham *et al.* 2007). Such multi-host/multi-pathogen systems are difficult to study in the wild because information on the full range of hosts is lacking, and because pathogens may interact differently with each other and each host species. Third, environmental perturbations can change the interplay between the host and pathogen and trigger disease outbreaks in ways that can only be understood through long-term monitoring. Fortunately in East Africa's Serengeti ecosystem, data on lions has been collected for over 40 years and lies within a framework of long-term studies of other predatory species, herbivores, human populations, and climatic conditions (Sinclair *et al.* 2008). Hence data collected from the Serengeti Lion Project provides the rare opportunity to tackle complex issues of disease dynamics in wild animal populations, with the ultimate aim towards conserving lions in their natural habitat.

This chapter will begin with an exploration of a wide variety of diseases in Serengeti lions. It will highlight differences between endemic and epidemic pathogens, show that pathogenicity is often difficult to discern and could vary by ecosystem (bovine tuberculosis), illustrate that some pathogens still fit the one- host, one-pathogen traditional disease model (feline immunodeficiency virus), and highlight that co-

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infections are not always harmful to the host (trypanosomes). The second part of the chapter will provide an in-depth case study on the dynamics of canine distemper virus in the Serengeti lion population, and will conclude with new research synthesizing biology and epidemiology through the use of detailed mathematical models.

Study system

The Serengeti Lion Project is an important study system for insights in infectious disease ecology, though it is best known for seminal research on lion social behavior, ecology and genetics (Schaller 1972, Bertram 1975, Bertram 1976, Bygott, Bertram & Hanby 1979, Pusey & Packer 1987, Packer *et al.* 1988, Packer, Scheel & Pusey 1990, Packer *et al.* 1991, Pusey & Packer 1994, Packer, Tatar & Collins 1998, Packer, Pusey & Eberly 2001, Packer *et al.* 2005). Continuous demographic data have been collected on lions residing in a 2000 km² area of the Serengeti National Park (Tanzania, East Africa) since March 1966 and in the 250 km² floor of the nearby Ngorongoro Crater since 1963 (Fig. 1) (Packer, Tatar & Collins 1998). In each Serengeti study pride one female is fitted with a radio collar, whereas the Crater lions are located by opportunistic sightings. The 25,000 km² Serengeti ecosystem is dominated by migratory ungulates that move with the seasonal rains (Sinclair 1995). The Serengeti lion population is estimated at around 3500 individuals and has considerable genetic diversity (Gilbert *et al.* 1991). In contrast, the Ngorongoro Crater provides a small oasis of persistent food and water (Hanby, Bygott & Packer 1995, Packer *et al.* 1999). The Crater population, currently comprises about 60 individuals, and enjoys consistently high food availability (Hanby, Bygott & Packer 1995, Kissui & Packer 2004). However, the Crater lions have passed through a population bottleneck of <15 adults with no immigration since 1969, and show considerable signs of inbreeding (e.g. sperm abnormalities, lack of genetic diversity) (O'Brien *et al.* 1987, Wildt *et al.* 1987, Packer *et al.* 1991).

While it has been feasible to study the entire lion population of the Crater floor, the Serengeti lion study area is restricted to the southeastern quarter of the region (Fig. 1). The Serengeti study area includes two contrasting habitats: woodlands that are dominated by *Acacia* and *Commiphora* trees and the open grass plains (Packer *et al.*

2005). Because lion prides are highly territorial, residential lions are not able to access the migratory prey year round. Hence, prides of lions residing in the woodlands have year-round access to residential prey, while the plains prides face 'feast or famine', depending on the location of the migratory herds (Scheel & Packer 1995). As a result, lions live at higher densities in the woodlands and lower densities on the plains (Scheel & Packer 1995, Packer *et al.* 2005).

Serengeti lions live in gregarious groups (prides) composed of one to 21 related females, their dependent offspring, and a resident coalition of one to nine males. Prides are territorial and infrequently contact their neighbours (Packer, Lewis & Pusey 1992); inter-pride encounters can be deadly (Schaller 1972, McComb *et al.* 1993, Grinnell, Packer & Pusey 1995). When prides grow too large, cohorts of young females split off and form a neighbouring pride (Pusey & Packer 1987) and are more tolerant of their non-pride relatives (VanderWaal, Mosser & Packer in press). Coalitions of males can be resident in more than one pride (Bygott, Bertram & Hanby 1979), and distribute their time between their various prides (Schaller 1972). In contrast, nomads are adult male and female lions that do not maintain a territory and move great distances through the ecosystem (Schaller 1972). Residents and nomads occasionally interact during mating, territorial defense, and at kills.

The disease facet of the Serengeti Lion Project benefits from a valuable archive of biological samples dating back to 1984, collected from individually identified, known-age lions. Lions are identified through natural marking (Pennycuick & Rudnai 1970), and each sample comes from a lion with a detailed life history. Information exists on individual ranging patterns, relatedness, and birth dates (normally accurate to one month), and contact patterns with conspecifics and other species can also be inferred. Blood is collected by tranquilizing an animal and then drawing blood, while fecal samples are collected during opportunistic sightings. The stored blood and/or fecal samples can then be used for retrospective surveys testing for a pathogen or exposure to a certain pathogen through the detection of antibodies (serology). Serological results can be interpreted as follows; by estimating the proportion of lions that were seropositive at a certain age in a particular year, age-seroprevalence curves can inform

whether diseases persist or are transient in the lion population (endemic vs. epidemic) (Packer *et al.* 1999). In addition, the timing of epidemic disease outbreaks can be estimated by plotting each year's annual seroprevalence rate (Packer *et al.* 1999). Because snapshot studies of exposure can be misleading, these data must be collected through the highs and lows in prevalence over prolonged periods (Cleaveland *et al.* 2007).

Endemic and epidemic diseases: viruses and parasites

Diseases can be classified as endemic or epidemic based on their persistence times in a population. Endemic diseases are consistently prevalent in a population and often demonstrate low virulence and long infectious periods (Anderson & May 1979).

Macroparasites are a classic example of endemic diseases. These are parasites in which the lifecycle usually occurs via transmission of free-living infective stages that pass from one host to the next. Macroparasites normally cause chronic morbidity in the host, instead of mortality (Hudson 2002). The Serengeti and Ngorongoro lion populations are infected with gastrointestinal endoparasites, which can be identified in faecal matter. In one study of 112 Serengeti and Ngorongoro Crater lions, 15 parasite taxa were identified (although three taxa were likely acquired from prey) (Muller-Graf 1995). Parasite species were aggregated in individual hosts, where a few lions were heavily infected and most lions were lightly infected (Muller-Graf 1995). This aggregation is consistent with other macroparasite studies in following the '20/80 rule' (20% of the population harbors 80% of the parasites) (Anderson & May 1991, Shaw, Grenfell & Dobson 1998, Hudson 2002). In a more detailed study of the cestode *Spirometra* spp. (the most common lion intestinal parasite), lions living in the Crater were more heavily infected than lions living in the Serengeti (Muller-Graf, Woolhouse & Packer 2000). It was difficult to assess whether these differences could be attributed to ecological differences (e.g. swampy vs. dry habitat, abundant vs. sparse prey) or from genetic differences (inbred vs. outbred). Cubs less than nine months in both locations were already heavily infected with *Spirometra* when sampled, and there were no significant correlations between individual parasite load and rainfall season, age or sex of the lion,

reproductive status, or pride size. In another cross-sectional study of 33 lions, over 19 species of parasites were identified in the lion population (although some were likely acquired from prey) (Bjork, Averbeck & Stromberg 2000). Again, the number of gastrointestinal parasite species per lion did not change significantly with respect to sex, habitat, or age of the lion.

The ecology of endemic diseases in natural populations is difficult to study without controlled experiments and intervention as it seems lions are constantly infected and the lion population and their gastrointestinal parasites likely remain near equilibrium. In order to detect the effects of genetic (inbred vs. outbred) or ecological factors (high vs. low prey densities) on levels of parasite infections, there would have to be a much wider array of study populations, covering a wider range of genetic or ecological factors (Muller-Graf, Woolhouse & Packer 2000). To assess whether macroparasites in any way ‘regulate’ the lion population, either lions or their parasites would have to be experimentally perturbed to detect any effects on morbidity and mortality (Tompkins *et al.* 2002). Long-term eradication/control of the lions’ gastrointestinal parasites would not be feasible and experimental infections would be impossible to justify.

In studies of disease in wildlife, it is often difficult to detect sick animals, locate carcasses for post-mortem exams, and to isolate the viral pathogens. In contrast, serological studies can be performed by screening large numbers of living individuals. However, serology merely determines whether an individual possesses antibodies to a certain pathogen and was therefore exposed *sometime* in the past; a positive serological result is unlikely to indicate current infection. As Serengeti lions have been known to live up to 20 years, a serological cross-sectional study could potentially be misleading. For example, the proportion of lions with antibodies for canine distemper virus (CDV) in 1985 could have been attributed to a constantly circulating (endemic) disease with increased cumulative exposure in adults, when, in fact, CDV had been absent from the population for several years, and the older age classes retained antibodies from an earlier epidemic (Fig. 2).

Feline herpesvirus (FHV) is a clear example of an endemic viral disease in the Serengeti lion population. Feline herpesvirus is a highly contagious respiratory disease spread by direct contact with an infected cat (Gaskell, Dawson & Radford 2006). Across a 10 year span, 372 out of 374 lions were positive for herpesvirus, including eight small cubs under one year old (Fig. 3) (Packer *et al.* 1999). As herpesvirus is consistently circulating and cubs are immediately infected, this is a pattern of chronic infection. Although FHV has been implicated in the death of a captive lion in Germany (Wack 2003), no signs of clinical disease have been attributed to FHV in the Serengeti or in other wild felid populations (Spencer & Morkel 1993, Packer *et al.* 1999, Driciru *et al.* 2006, Ramsauer *et al.* 2007). However, since 100% of the Serengeti population is infected, it is difficult to compare infected and uninfected hosts to assess potential impacts of infection status on fecundity or survival (Packer *et al.* 1999).

In contrast to endemic diseases, epidemic diseases cause distinct rises and falls in patterns of seroprevalence, often briefly infect a population, and have the potential to inflict high mortality (May & Anderson 1979). Epidemic viruses frequently sweep through a population, burn out because of lack of susceptibles, and then invade again once the susceptible population increases to a critical density threshold. Coronavirus, parvovirus and calicivirus are all epidemic viruses in the Serengeti lion population, showing periods of high exposure, followed by 4-9 yrs of declining seroprevalence before another period of high exposure (Fig. 4) (Packer *et al.* 1999). Whereas endemic diseases continuously infect the youngest age classes (Figs. 10.3 & 10.6), epidemics can best be identified from temporal gaps in infection in exposure in the youngest age classes (Fig. 4). For example, feline coronavirus (FCoV) is spread by indirect faecal-oral routes (and possibly aerosolized routes), can infect domestic dogs, and can turn into the more pathogenic feline infectious peritonitis in domestic cats (Addie & Jarrett 2006). FCoV was found in extremely low levels in South African and Namibian lions (Spencer 1991, Spencer & Morkel 1993), but was found in 57% of Serengeti lions (Hofmann-Lehmann *et al.* 1996). In Serengeti lions, coronavirus serostatus varied significantly across years in juveniles, but not in adults (Fig. 4a). Young lions in Ngorongoro Crater were positive for coronavirus, but the sample size was too small to

detect variation among years. Using the unique combination of age and serology data, the timing of a coronavirus epidemic in Serengeti could be inferred by comparing the seroprevalence data with the age of each study animal. For example, of all lions sampled between 1984 and 1988, only those animals born in July 1984 or before were seropositive for coronavirus (Fig. 5b). Packer *et al.* 1999 interpret this to mean that 1984 was the end-date for an epidemic; other coronavirus end-dates were estimated in 1988 and 1993 (Fig. 4a).

Similar to patterns of coronavirus exposure, parvovirus (FPV/CPV) seroprevalence in adults did not differ significantly across years, but varied significantly in younger animals (Fig. 4b). Parvovirus is a multi-host virus most commonly spread by indirect contact through environmental contamination, where parvovirus can remain infectious up to a year at room temperature (Greene & Addie 2006). Parvovirus is a suspected cause of wolf pup (*Canis lupus*) mortality (Mech & Goyal 1993), but does not seem to cause morbidity or mortality in lions. An age-seroprevalence curve for lions tested between 1984 and 1991 revealed that the last period of exposure in Serengeti lions during this time frame was in 1985 (Fig. 5a), with other likely outbreaks ending in 1976 and 1992 (Fig. 4b) (Hofmann-Lehmann *et al.* 1996, Packer *et al.* 1999).

Finally, feline calicivirus (FCV) is an upper respiratory infection similar to FHV, is spread by direct contact, and some strains can cause high mortality in domestic kittens (Gaskell, Dawson & Radford 2006). When calicivirus seroprevalence was plotted by year of birth for lions sampled between 1991 and 1994, no lion born after March 1990 was positive, indicating that 1990 was a likely end-date for a calicivirus epidemic (Fig. 5c), with other likely end-dates of 1980 and 1985 (Fig. 4c). Like other serological studies of lions, there were no consistent signs of clinical disease, excess mortality or decreases in lion fecundity due to infections from coronavirus, parvovirus or calicivirus (Spencer 1991, Spencer & Morkel 1993, Hofmann-Lehmann *et al.* 1996, Packer *et al.* 1999, Driciru *et al.* 2006).

A low impact pathogen or an insidious threat: bovine tuberculosis

Mycobacterium bovis, the causative agent of bovine tuberculosis (bTB), is a bacterium of growing concern in African wildlife (Michel *et al.* 2006). In Kruger National Park, South Africa, an epidemic of bTB infection in African buffalo (*Sycerus caffer*) has been moving northwards across the park since 1990 (Michel *et al.* 2006). Monitored lions from the same area have become infected (Cleaveland *et al.* 2005, Michel *et al.* 2006), and although the Kruger lion population currently seems stable (Ferreira & Funston, in press), both lions and buffalos show mortality and morbidity. In contrast, between 1985 and 2000 none of the 19 lions sampled in the Ngorongoro Crater were seropositive for bTB and only eight of 184 (4%) Serengeti lions were seropositive for bTB (Cleaveland *et al.* 2005). The Tanzanian samples were collected over a period of 15 years and, although one of the positive lions was sampled in 1984, there were no significant differences between average prevalences from 1984-1996 and 1997-2000. This indicates that bTB has been rare in the population for a long time, and is not spreading quickly. While clinical signs were seen in four out of eight seropositive animals, and seropositive animals survived for a shorter (but non-significant) amount of time than did non-infected individuals, with such a low sample size of positives, it is difficult to quantify the pathogenicity of bTB in Serengeti lions (Cleaveland *et al.* 2005). Cleaveland *et al.* suspect that exposure in lions was due to eating infected prey.

One-host, one-pathogen: feline immunodeficiency virus

Serengeti lions are infected with a lentivirus, feline immunodeficiency virus (FIV), which is genetically homologous and functionally analogous to human immunodeficiency virus (HIV) (Brown *et al.* 1994). Like HIV, once infected, FIV permanently infects the host. FIV has species-specific strains, and the strain infecting lions is named FIV-Ple (Olmsted *et al.* 1992). In contrast to recent work showing frequent FIV transmission from bobcats to pumas in the United States (Franklin *et al.* 2007), phylogenetic analysis suggest that cross-species transmission is unlikely between lions and other large African carnivores (Troyer *et al.* 2005).

Although lions have been likely hosts to FIV since the late Pleistocene (Brown *et al.* 1994, Pecon-Slattery *et al.* 2008), not all populations of lions are currently infected. African lions in Namibia and Asiatic lions in India test negative for FIV. On the other hand, FIV is extremely prevalent in East African and South African lions, and the incidence in these populations is higher than in any other wild or domestic felid population (Olmsted *et al.* 1992, Driciru *et al.* 2006). Serengeti lions not only have exceptionally high incidence of FIV (84-93%), but because these high levels are consistently maintained over many years, FIV is endemic in the Serengeti (Olmsted *et al.* 1992, Brown *et al.* 1994, Hofmann-Lehmann *et al.* 1996, Packer *et al.* 1999, Troyer *et al.* 2005). FIV infection rates do not change significantly by sex or across years and this is true for both adult and juvenile lions (Packer *et al.* 1999). Although not all juvenile lions test positive, the vast majority of lions in Serengeti and Ngorongoro are infected by four years of age. Because the entire adult Serengeti and Ngorongoro population is FIV-positive, there is no control group in which to assess effects of infection status on fecundity (Packer *et al.* 1999).

There are differences in rates of infection with respect to habitat type; lions inhabiting the Serengeti plains show lower rates of infection than lions inhabiting the woodlands or Ngorongoro Crater (Fig. 6) (Packer *et al.* 1999). Prides in the three habitats live in varying densities and have different within- and between-pride contact patterns. While all of these factors can influence infection rates, the differing rates of infection in various habitat types are unexplained.

Although FIV-Fca causes immunosuppression and mortality in domestic cats (*Felis silvestris catus*) (Yamamoto *et al.* 1988, Ackley *et al.* 1990), there are no obvious signs of immunodeficiency or disease in Serengeti lions, nor in other wild feline species (Olmsted *et al.* 1992, Roelke-Parker *et al.* 1996, Packer *et al.* 1999, Ramsauer *et al.* 2007). FIV infection had no age or sex-specific effects on determining host longevity (Hofmann-Lehmann *et al.* 1996, Packer *et al.* 1999). Even FIV co-infection with other viruses (i.e. canine distemper virus, feline herpesvirus, feline calicivirus, feline parvovirus, and feline coronavirus) did not reduce host longevity (Packer *et al.* 1999).

Lions can be co-infected with different strains of FIV. Six FIV-Ple strains or subtypes occur throughout Africa (Antunes *et al.* 2008). These subtypes (based on *pol* gene sequence divergences) come from a common FIV-Ple lion ancestor, but are distinct from each other (Brown *et al.* 1994). There is high genetic diversity within and between the subtypes (Brown *et al.* 1994). Three different clades are present in the Serengeti population (Brown *et al.* 1994), and multiple subtypes are found within the same pride, and within the same individual (Troyer *et al.* 2004). In a recent study, 43% of FIV-positive individuals in the Serengeti were infected with multiple strains of FIV (Troyer *et al.* 2004). Thus co-infection with one subtype of FIV does not necessarily confer immunity against secondary infection from another subtype (Troyer *et al.* 2004).

It is unknown whether FIV is spread primarily through horizontal transmission (neighbour-to-neighbour) or from vertical transmission (parent-offspring). Both in domestic cats and in lions, horizontal transmission seems to be the major route of infection and likely occurs during biting (Yamamoto *et al.* 1988, Brown *et al.* 1994). As evidence of horizontal transmission, two male Serengeti lions born in 1982 and in 1986 both tested negative for FIV in 1987 but tested positive in 1989 (Brown *et al.* 1994). In addition, FIV-positive cubs were born from FIV-negative mothers, and FIV-negative cubs were born from FIV-positive mothers (Brown *et al.* 1994). There was evidence of both between-pride and within-pride transmission though phylogenetic analysis of sequences (Troyer *et al.* 2004). The strains were well mixed across all prides: six out of 13 prides were infected with all three strains. In contrast, one pride showed evidence of monophyletic clustering. Although closely-related lions often had closely-related viral sequences (e.g. three sibling pairs and a mother-daughter pair), indicating a common viral ancestor, it is hard to determine if these individuals were infected by each other (vertical transmission in case of the parent-offspring) or by another lion (Troyer *et al.* 2004). The identification of transmission routes can be difficult because closely related lions are often found in close association with each other.

According to phylogenetic analyses, lions have been infected with FIV-Ple virus for long periods of time, and the three FIV subtypes diverged a long time ago—maybe

as far as the radiation of the genus *Panthera* (Olmsted *et al.* 1992, Brown *et al.* 1994). Because there is no evidence for immune pathology or mortality from this ‘ancient’ virus, the interactions between lions and FIV-Ple could be an example of ‘modern symbiosis’ or commensalism between a host and pathogen (Olmsted *et al.* 1992, Brown *et al.* 1994). This attenuation makes the FIV-Ple host-virus relationship a great contrast to the high pathogenicity of the recent HIV epidemic (Carpenter & O'Brien 1995).

Co-infections are not always harmful: trypanosomes

Trypanosomes are protozoan parasites that cause substantial economic and public health problems in sub-Saharan Africa due to the morbidity and mortality of ‘sleeping sickness’ in humans and livestock (Schmunis 2004, Shaw 2004). Because trypanosomes have extremely complex antigenic surface proteins that change composition every 3-5 days, they can evade attack by the host’s immune system (Morrison *et al.* 2005).

Trypanosomes have a long history of infection in the Serengeti; in fact, the presence of trypanosomiasis influenced the initial gazettement of the Serengeti National Park. The tsetse fly (*Glossina* spp.), the vector that transmits the disease, kept the Serengeti uninhabitable for humans and their livestock, yet available for wildlife (Matzke 1979). Sleeping sickness still circulates in the Serengeti, as seen in 2001 when 23 people died from trypanosome infection (Jelinek *et al.* 2002, Mlengeya *et al.* 2002).

Trypanosomiasis is thought to be maintained in livestock in other parts of sub-Saharan Africa (Welburn *et al.* 2001) and in wildlife populations in the Serengeti (Kaare *et al.* 2007).

Lions are likely infected with trypanosomes in one of two ways: through the bite of an infected tsetse fly, or via oral inoculation when eating infected prey. Tsetse flies need shade and woody vegetation to survive and thus do not inhabit the plains or the Crater. In a study of 123 Serengeti and Ngorongoro lions, trypanosomes were found by microscopy in 32 individuals (Averbeck *et al.* 1990). While trypanosome prevalence did not vary with respect to the lion’s age or sex, prevalence was highest in the Serengeti woodlands, lower in the Serengeti plains and absent in the Crater (Table 1). Prevalence of trypanosome infection correlated with increasing levels of tsetse flies

(Table 1), suggesting infection directly from tsetse bites. Two out of 29 plains lions, however, did get infected, suggesting that lions might also be inoculated by consuming infected prey that migrates to the plains from the woodlands. Prey such as wildebeest (*Connochaetes taurinus*), grants gazelle (*Gazella granti*), Thompson gazelle (*Gazella thomsonii*), and warthogs (*Phacochoerus aethiopicus*) are known to be infected with trypanosomes (Baker 1968, Kaare *et al.* 2007). However, plains lions sometimes make short forays to tsetse fly habitat during droughts (Averbeck *et al.* 1990, Packer, Scheel & Pusey 1990), making it difficult to infer the mode of trypanosome transmission.

While microscopy could not identify trypanosomes to the species level, molecular techniques have identified multiple *Trypanosoma* spp. that co-infect the Serengeti lions: *Trypanosoma congolense*, *T. brucei rhodesiense*, the causative agent of human sleeping sickness, and the non pathogenic *T. brucei brucei* (Welburn *et al.* 2008). Welburn *et al.* identify different age-prevalence patterns of exposure to *T. brucei* and *T. congolense* (Fig. 7). Lions are rapidly exposed at a young age to *T. brucei*, and then prevalence decreases, while prevalence of *T. congolense* increases steadily with age. Because *T. congolense* is more common, and more genetically diverse than *T. brucei*, Welburn *et al.* conclude that *T. congolense* infection confers protective immunity against infection with *T. brucei*. In addition, cross-immunity likely explains why lions are not infected with the human-pathogenic *T. brucei rhodesiense* after the age of six. Lions do not show increased mortality due to infection. Welburn *et al.* show the first evidence of acquired immunity to natural infection for trypanosomes, and more broadly, this study is a useful way to rethink the assumption that all co-infections necessarily harm the host.

Case study: canine distemper virus

Co-infection increases virulence in a multi-host pathogen

Infectious disease was not a major research focus when the Serengeti Lion Project was founded in 1966. However, things changed in 1994 with the observation of six lions experiencing violent symptoms such as *grand-mal* seizures and three lions with

myoclonus (recurrent twitching) (Roelke-Parker *et al.* 1996). Over a period of eight months, one-third of the study lions died; a huge deviation from normal mortality rates, and a sign of a previously unappreciated threat from infectious disease (Roelke-Parker *et al.* 1996). When canine distemper virus (CDV) was identified as the causative agent, it was the first time that CDV had been detected in wild lions (Appel & Summers 1995). At the end of the outbreak, CDV had spread extensively across the Serengeti ecosystem, infecting 85% of survivors (Roelke-Parker *et al.* 1996).

Domestic dogs (*Canis familiaris*) were the likely source of infection into the lion population. In 1992 and 1993, CDV was circulating in the high-density domestic dog population to the northwest of the park and was not present elsewhere in the ecosystem (Fig. 8a) (Roelke-Parker *et al.* 1996, Cleaveland *et al.* 2000). While it made intuitive sense that domestic dogs were the source of CDV, the exact mechanism of transmission between dogs and lions remained a mystery. As CDV is transmitted by aerosol or droplet exposure (or possibly by eating an infected carcass) (Appel 1987, Greene & Appel 2006), and domestic dogs and lions do not occupy the same habitat, it seemed unlikely that a dog could transmit CDV directly to a lion, suggesting an intermediate link, such spotted hyaenas, which are known to ‘commute’ long distances and to enter agricultural areas outside the national park (Hofer & East 1993b). Another question remained unanswered: was this the first time that lions had been exposed to CDV?

A retrospective serological study showed discrete periods of CDV exposure in the study population (as evidenced by declining CDV seroprevalence levels in the 1980s reflecting earlier exposure possibly from a 1981 outbreak), although no symptoms or excess mortality were observed during these earlier periods (Roelke-Parker *et al.* 1996, Packer *et al.* 1999). Why then was the 1994 outbreak so harmful to the lion population? Was this simply a new, more virulent strain of CDV (Packer *et al.* 1999)?

Then 40% of Crater lions died in 10 weeks in 2001, and 10 out of 10 sampled lions tested positive for CDV antibodies (Kissui & Packer 2004, Munson *et al.* 2008). Retrospective serological results of stored lion samples showed that Ngorongoro lions were exposed to distemper at least once in the past (before 1984, most likely 1980), but

had not died or shown symptoms in the earlier period (Packer *et al.* 1999). In total, out of at least seven CDV outbreaks in the Serengeti and Crater lion populations since 1975, lions only experienced symptoms and high mortality in the Serengeti in 1994 and in the Crater in 2001.

New results indicate that the two periods of mass mortalities were due to a convergence of biotic and abiotic conditions to create a ‘perfect storm’ where CDV exacerbated the impacts of a tick-borne pathogen (Munson *et al.* 2008). Lions are consistently infected with low levels of *Babesia*, a tick-borne parasite that can be transferred from herbivores. Severe droughts led to large-scale starvation and mass mortalities in African buffalo (*Syncerus cafer*) in the Serengeti in 1993 and the Crater in 2000; the weakened buffalo reached unprecedented heights in the lions’ diet and exposed the lions to high levels of *Babesia* infection. CDV is immunosuppressive, and the outbreaks in early 1994 and early 2001 allowed already high levels of *Babesia* to overwhelm the co-infected lions. Serengeti prides in 1994 showed no increase in mortality if they were only exposed to CDV or only to high levels of *Babesia* (Munson *et al.* 2008).

Levels of *Babesia* were consistently higher in the Crater than the Serengeti. Fyumagwa *et al.* 2007 trace the build up in Ngorongoro Crater to the 1970s (Fig. 9) when management authorities embarked upon a policy of fire suppression and evicted the pastoralist Masai from the Crater floor, removing the effects of fire, allowing the grass to grow taller and increasing tick survival. Meanwhile, the buffalo population grew in size, and hence the numbers of tick-infested buffalos also increased, especially during the El Niño wet years (1997/98). This was followed by the drought of 1999/2000, which caused the death of buffalos, wildebeest, and rhinos, and the consequent die-off in the Ngorongoro lion population (due to disease rather than drought) (Fig. 9) (Fyumagwa *et al.* 2007). Although the Serengeti lion population was large enough to return to its original population size by the middle of 1997 (Packer *et al.* 1999, Packer *et al.* 2005), frequent outbreaks of disease seem to have kept the Crater population below carrying capacity for the past 14 years (Kissui & Packer 2004).

Integrating biology and epidemiology into models

In order to manage disease threats effectively (e.g. to prevent CDV/*Babesia* from causing mass mortalities again), it is important to understand which populations maintain multi-host pathogens in the greater Serengeti ecosystem (Cleaveland *et al.* 2007, Cleaveland *et al.* 2008). The maintenance population is the species, or set of species, in which the infection can independently persist (Haydon *et al.* 2002). In light of this goal, a mass vaccination program was initiated in 2003, vaccinating >35,000 domestic dogs per year for CDV, parvovirus, and rabies with an aim of reducing disease transmission to Serengeti wildlife (Cleaveland *et al.* 2007). By 2008, the program appeared to have successfully eliminated canine rabies from wildlife in the Serengeti ecosystem (Lembo *et al.* 2008) and it is not yet clear whether there has been any impact on parvovirus exposure in the lions; however, CDV struck the Serengeti lions in 2006 (Munson *et al.* 2008).

Because CDV still seems to be a threat to lions (despite the dog vaccinations), identification of a maintenance population is crucial. Some researchers claim that CDV can be maintained solely within the lion population (without transmission from other species such as hyenas or jackals), fuelled by occasional spill-over from the domestic dog population (Guiserix *et al.* 2007). If so, lion-to-lion transmission alone should account for the observed dynamics of the 1994 CDV outbreak inside the Serengeti National Park. To test this hypothesis, an empirically-parameterized network model was constructed to represent the demographic, spatial, and contact structure of the Serengeti lion population before the 1993/4 outbreak (Craft *et al.*, in prep). In contrast to Guiserix's model, where all lions in the ecosystem have an equal chance of contacting other lions, the network model explicitly defines the different lion social groups and assigns contacts between groups according to network adjacencies.

Lion network and contact structure

The observed population structure and contact patterns of the Serengeti lions were estimated using empirical data from the Lion Project (Table 2). The network model placed $N_p = 180$ prides and $N_N = 180$ coalitions of nomads at random locations in an

$A = 10,000 \text{ km}^2$ region of the Serengeti. Prides were assigned to be adjacent according to the estimated adjacency model (M_{adj}). A fraction of adjacent pairs of prides (Ψ) were randomly assigned to have recently split. Each pride was given a group size (X_p) drawn from an empirical distribution. Contacts between prides occurred at an average of $C_p = 4.55$ contacts per two-week period per pride, as estimated from a study in which 16 lionesses were observed continuously for a total of 2213 hours (Packer, Scheel & Pusey 1990, Scheel & Packer 1991). Contacts between pairs of prides occurred stochastically at rates weighted by a logistic function of the network distance between the centroids of their territories (M_{contact}).

Coalitions of resident males and nomads were treated separately from prides of females and cubs. Male coalitions were represented as single units that increase connectivity between prides. Each territorial coalition belonged to either one or two prides; an estimated fraction η of all prides shared their territorial coalition with one of their adjacent prides, and every other pride had a territorial coalition to itself. If a territorial coalition was associated with more than one pride, it would switch between prides according to ζ , the territorial male migration rate. Nomadic lions were assigned group sizes (X_N) averaging 1.5 members and were assumed to migrate via a variance gamma process (M_{nomad}) as estimated from a GPS-collared nomad (Fig. 10). Nomads were assumed to contact their local pride according to the average rate of pride-nomad contacts per pride (C_N).

When a pride contacted another pride or nomadic coalition, only a subset of the pride was involved in the interaction (G), and the number of lions involved depended on the size of that pride. When nomads contacted prides, all members of the coalition were assumed to be present. Inter-group contacts of resident males were incorporated into the pride contact patterns.

Epidemiological model

In this network model, prides move through each susceptible, exposed, infectious, and recovered class as a unit; prides contact other prides as a function of their distance

within territory adjacency networks; male coalitions transmit disease between their residential prides; and nomads migrate and contact prides according to empirically-estimated rates. CDV was introduced into this network and was transmitted among prides according to incubating/infectious parameters estimated from the domestic dog literature. Simulations were run across a range of transmissibility values (probability that an infection is passed during a contact between a susceptible and infectious individual). Model output was compared to three characteristics of the 1994 outbreak: (1) 17/18 study prides were infected, (2) infection spread in a discontinuous pattern through the study area (Fig. 8c), and (3) CDV took 35 weeks to spread 100 km to the Maasai Mara National Reserve (MMNR) (Cleaveland *et al.* 2007, Craft *et al.* 2008).

Nomads—are they superspreaders?

Although nomads are numerous, travel long distances, and are likely candidates to be considered superspreaders (Lloyd-Smith *et al.* 2005), their impacts on model CDV disease dynamics were surprisingly low. In fact, for extensive outbreaks with 95% prevalence, nomads only accounted for 10% of all transmissions, whereas the vast majority of transmissions were pride-to-pride (neighbors, 53.1%; second degree neighbors, 27.8%, and third degree neighbors 8.3%) and prides four prides away or greater and shared males only accounted for less than 1% of transmissions. To assess the effects of nomads on CDV prevalence, spatial spread, and velocity, simulations were run where nomads migrated at an unrealistically fast rate and were removed altogether. In the simulations (regardless of the presence or migration rate of nomads) it was possible to infect at least 95% of prides, as seen in 1994. Accelerating the migration rate of nomads only slightly increased overall CDV prevalence among prides and removing nomads from the simulations slightly decreased overall prevalence (Fig. 11). The spatial spread of CDV, driven by pride-to-pride transmission, was wave-like throughout the ecosystem and when we either increased the nomad migration rate or removed nomads from the simulations, the overall spread in the population remained wave-like, however was correlated at longer network distances with the high nomad migration rate (Fig. 12). Finally, the model results produced a wave of CDV that

traveled at a velocity consistent with the observed velocity. However, because there was no difference in the velocity of the epidemic when nomads were removed from the simulations, this again showed that nomads were not driving the spatial spread (Fig. 13). For diseases with relatively short infectious periods, like the two weeks for CDV, nomads do not appear to be superspreaders.

Did lions maintain the 1994 CDV outbreak themselves?

Model results showed that the observed 1994 CDV spatial spread pattern and velocity were likely to occur at low transmissibilities, while the observed prevalence was likely at higher transmissibilities, and only a select few simulations exhibited both the observed prevalence and velocity (Fig. 14). The results from the model suggest that epidemics could not have been as large and as slow as the observed 1993-94 outbreak; hence the lion-to-lion transmission model lacked a critical component of the actual transmission dynamics. Lions could not maintain distemper on their own, and the missing piece of transmission was presumably multiple introductions of disease from other wild carnivore species, such as spotted hyaenas (*Crocuta crocuta*) and jackals (*Canis spp.*).

It is reasonable that other carnivores were involved in the fatal Serengeti outbreak, as all families in the order Carnivora are susceptible to CDV (Williams 2001), and lions frequently interact with hyaenas and jackals at carcasses (Cleaveland *et al.* 2008). A multi-host explanation for the observed CDV dynamics is also consistent with (a) a genetic analysis of a single CDV variant found in lions, hyaenas, bat-eared foxes (*Otocyon megalotis*), and domestic dogs at the time of the epidemic (Haas *et al.* 1996, Roelke-Parker *et al.* 1996, Carpenter *et al.* 1998); (b) observations of a few sick carnivores at the time of the epidemic (but no known effects on hyaena or jackal populations) (Roelke-Parker *et al.* 1996); (c) serological reconstruction of an epidemic in hyaenas, dogs, and lions (Fig. 8b) (Kock *et al.* 1998, Harrison *et al.* 2004); and (d) the concept of morbilliviruses requiring a much larger critical community size than 3,000 lions (Bartlett 1960, Grenfell, Bjornstad & Kappey 2001). In other words, the 1994 CDV epidemic observed in lions was likely fuelled by multiple carnivore species.

Multi-host dynamics

If lions could not produce the observed CDV outbreak, and other wild carnivores were feasibly involved in transmission to the lion population, could a multi-host spatial model account for the patchy pattern of CDV spread seen in lions in 1994 (Fig. 8c)? To test this hypothesis, a stochastic susceptible-infected-recovered multi-host model was constructed which allowed transmission between a highly territorial species, like lions, and 1-2 more gregarious hosts, such as hyaenas and jackals (Craft *et al.* 2008). Social structure of each species was explicitly modeled by varying within- and between-group transmission rates (e.g. isolated vs. well connected territorial structures) while interspecific transmission with sympatric carnivores occurred at both low and high rates. According to model results, when other gregarious species were coupled with lions at low transmissibility, the erratic and discontinuous patterns of CDV spatial spread were similar to those seen in lions in 1994 (Craft *et al.* 2008). Based on this simplified model, it is difficult to identify which carnivore species were likely involved in repeat transmission into the lion population, but rather that low interspecific contact rates could have accounted for the high prevalence and erratic spatial spread of CDV seen in 1994 in the lion population.

The results of both the network and the multi-host models, in combination with the observational and viral work, suggest that lions are a non-maintenance population for canine distemper virus, and because lions cannot independently maintain chains of CDV transmission, CDV control efforts should focus on other carnivores besides lions. Domestic dogs are a likely maintenance population for CDV, but whether other wild carnivores are part of this maintenance population remains unknown.

Conclusions

Even within large well-protected areas like the Serengeti, species like lions can be threatened by infectious disease (Cleaveland *et al.* 2007). These diseases can originate from outside the protected area, and outbreaks can be triggered by climatic factors. As we expect more climatic extremes from global climate change, this could have

unexpected effects on disease dynamics in wild animal populations (Munson *et al.* 2008). Disease dynamics are complex and understanding them requires coordinated and integrated ecosystem-level approaches (Cleaveland *et al.* 2008). In order to conserve free-ranging lions, and wild felids in general, we need to effectively integrate veterinary epidemiology into carnivore conservation and management, and focus our efforts on long-term, integrative, cross-species, cross-pathogen research (Cleaveland *et al.* 2007).

Which management approach should be adopted to protect wild felids from infectious disease threats? It is logistically infeasible to protect all cats from all diseases—some diseases are non-pathogenic and resources are limited. For a start, it is important to understand the potential impacts of disease on long-term population viability (Driciru *et al.* 2006). Ironically, this does not necessarily mean the total elimination of a pathogen from a system. Studies have shown that depending on reservoir dynamics and resource availability, instead of attempting to eliminate a disease, prevention of the largest outbreaks that would decrease population numbers below a viable threshold may be more practical (Vial *et al.* 2006, Cleaveland *et al.* 2007). So how do we prioritize which diseases, and in which situations, to focus our efforts? Maybe we should focus interventions on diseases that are of anthropogenic origin (i.e. viruses associated with humans and their domestic dogs like rabies, CDV, and parvovirus) and focus concerted effort on small, fragmented populations that might not recover from a decline in population size due to disease. Specifically, what lessons can we learn from the Serengeti Lion Project's disease studies?

First, studies of disease dynamics in Serengeti lions show that endemic diseases like gastrointestinal macroparasites, FIV, and FHV can persist in low-density or small populations, such as the small population of Crater lions and the low-density lions on the Serengeti plains. On the other hand, epidemic diseases either need a large number of susceptibles in order to persist (FCV), or the ability to infect a suite of hosts (CDV, FPV/CPV, FCoV). Ecological studies in Serengeti lions also illustrate that co-infection can either lessen or increase virulence, as seen with examples from trypanosomes and CDV/*Babesia*.

Secondly, disease status should be considered in lion relocations, as different viruses are present in different populations, as seen when comparing the nearby lion populations of Ngorongoro Crater and Serengeti (Hofmann-Lehmann *et al.* 1996). In addition, FIV, FHV, and rarely FCV and FPV/CPV infections can persist in seropositive hosts and asymptomatic carriers can continue to transmit, or shed, the virus (Driciru *et al.* 2006, Gaskell, Dawson & Radford 2006). A translocation could turn into a conservation disaster if a shedding individual was introduced into a totally susceptible population.

Finally, we have learned that in the Serengeti some diseases are harder to control than others. This is likely related to the concept of R_0 (the number of secondary infections produced by one infectious individual in a completely naïve population). Through the domestic dog vaccination campaign, Hampson has demonstrated that because the R_0 for rabies is surprisingly low (around 1.1-1.2) the elimination of canine rabies is logistically feasible (Hampson 2007). On the other hand, despite extensive dog vaccinations, Serengeti lions were still exposed to CDV in 2006 (Munson *et al.* 2008). If CDV is similar to other morbilliviruses like measles with its high R_0 (Lloyd-Smith *et al.* 2005), then CDV is more contagious than rabies. If we want to eliminate CDV to protect lions and other carnivores, we would likely need to increase vaccination coverage of domestic dogs (and other carnivores?). However, it may be that the total elimination of CDV from the ecosystem is not practical, and efforts should instead be placed on protecting small, fragmented populations, like wild dogs, from CDV. Alternatively, if we wanted to protect an isolated population of lions from excess mortality from CDV/*Babesia* co-infection, instead of focusing on the CDV, we could reduce lion tick load by keeping levels of ticks to a minimum in the ecosystem, as the Ngorongoro Crater authorities are currently doing with controlled burns (Fyumagwa *et al.* 2007).

Tables & Figures

Table 1. Prevalence of trypanosome infection in lions as detected by microscopy in four habitat types of Serengeti and Ngorongoro in order of decreasing occurrence of tsetse flies (see Fig. 1 for geographic locations) (Adapted from Averbeck et al. 1990).

Lion habitat type	Occurrence of tsetse flies	Prevalence (%) of <i>Trypanosoma</i> spp.
Serengeti Woodlands	Common	50 (26/52)
Serengeti Woodlands/Plains Border	Rare	11 (3/28)
Serengeti Plains	Absent	7 (2/29)
Ngorongoro Crater	Absent	0 (0/10)

Table 2. (a) Lion demographics. (b) Contact parameters. (From Craft *et al.* in prep)

Demographic parameters	Estimated quantities	Distributions
A : area of ecosystem	10,000 km ²	N/A
N_p : number of prides in ecosystem	180	$U(150,200)$
X_p : pride sizes (number of females and cubs over three months old)	X_p : Gamma(k, θ) with $\theta = 4.707$, $k = 2.226$ Mean pride size = 10.48	θ : $N(4.707, 1.243)$ k : $N(2.226, 0.636)$
η : fraction of prides that share territorial males with one other pride	0.882	η : $N(0.882, 0.078)$
ζ : rate at which territorial male coalitions switch prides	0.25 switches/day	ζ : $N(0.25, 0.070)$
M_{adj} : territory adjacency model	$\ln\left(\frac{P_{adj(AB)}}{1 - P_{adj(AB)}}\right) = 1.483 - 0.386 \cdot S_{AB}$ (S_{AB} = the number of prides located in the joint radius of A and B) Mean number adjacent prides = 7.36	intercept $\sim N(1.483, 0.225)$ slope $\sim N(-0.386, 0.041)$
Ψ : proportion of adjacent prides recently split from a common pride	0.063	$\Psi \sim N(0.063, 0.021)$
N_N : # nomads	180	$U(150-200)$
X_N : nomad group sizes	X_N : Log-normal(μ, σ) with $\mu = 0.292$, $\sigma = 0.446$ Mean group size = 1.51	$\mu \sim N(0.292, 0.065)$ $\sigma \sim N(0.446, 0.046)$
M_{nomad} : nomad movement model Horizontal (x) and vertical (y) displacements per day are given by gamma distributions	Disp _x : Gamma _x (k_x, θ_x) with $k_x = 0.382$, $\theta_x = 2.85$ Disp _y : Gamma _y (k_y, θ_y) with $k_y = 0.714$, $\theta_y = 1.743$	$k_x \sim N(0.382, 0.029)$ $\theta_x \sim N(2.85, 0.02)$ $k_y \sim N(0.714, 0.029)$ $\theta_y \sim N(1.743, 0.019)$

Contact parameters		
C_p : average rate of pride-pride contacts per pride	4.55 contacts/two weeks	$C_p : N(4.55, 0.573)$
M_{contact} : contact weighting model	$\ln\left(\frac{w_{c(A,B)}}{1 - w_{c(A,B)}}\right) = \alpha + \beta_d d_t(A,B) + \begin{cases} -\beta_s & \text{if recently split} \\ \beta_s & \text{otherwise} \end{cases}$ <p>$w_{c(A,B)}$ is the weighting factor for the contact rate between A and B and $d_t(A,B)$ is the territory distance between the prides.</p>	
α : intercept	3.265	$\alpha : N(3.265, 0.371)$
β_d : network distance coefficient	1.698	$\beta_d : N(1.698, 0.264)$
β_s : recent split coefficients	0.696	$\beta_s : N(0.696, 0.220)$
C_N : average rate of pride-nomad coalition contacts per pride	7.136 contacts/two weeks	$N(7.136, 1.018)$
G : pride group size during contact $G' = \log(G + 1)$	$G' \sim N(\mu_{G'}, \sigma_{G'})$ with $\mu_{G'} = 0.447 + 0.014 \cdot X_p,$ $\sigma_{G'} = 0.232$ Mean group size = 3.65	$\mu_{G'}$ intercept $\sim N(0.447, 0.057)$ $\mu_{G'}$ slope $\sim N(0.014, 0.004)$ $\sigma \sim N(0.232, 0.022)$

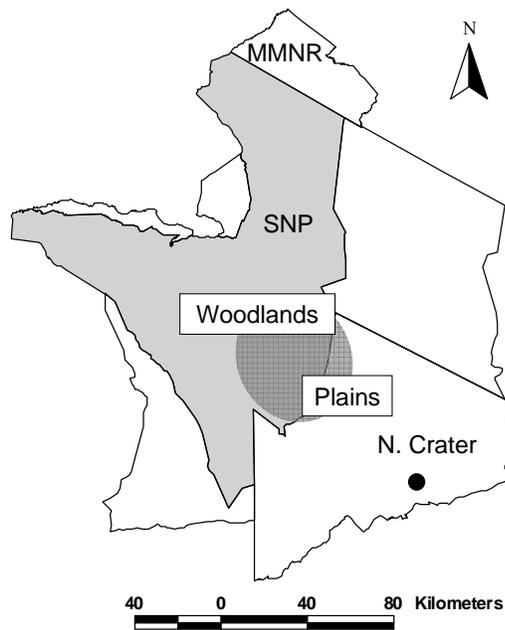


Figure 1. Map of Serengeti National Park (SNP) and the surrounding protected areas in East Africa, including the Masai Mara National Reserve, Kenya (MMNR) and Ngorongoro Crater (N. Crater). Lions have been studied continuously inside the Ngorongoro Crater (black circle) and in the SNP (gray oval) since the 1960s. The Serengeti study area is divided into two habitat types: woodland in the north, and plains to the south.

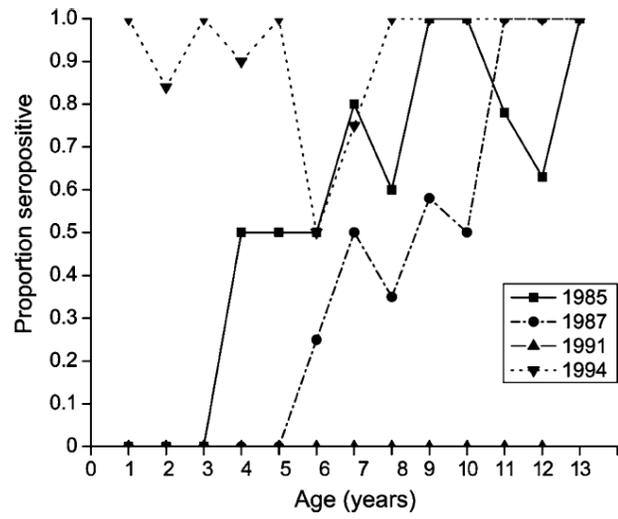


Figure 2. Canine distemper virus age-seroprevalence patterns in the Serengeti lion study population from cross-sectional samples in 1985, 1987, 1991, and 1994. (From Cleaveland *et al.* 2007)

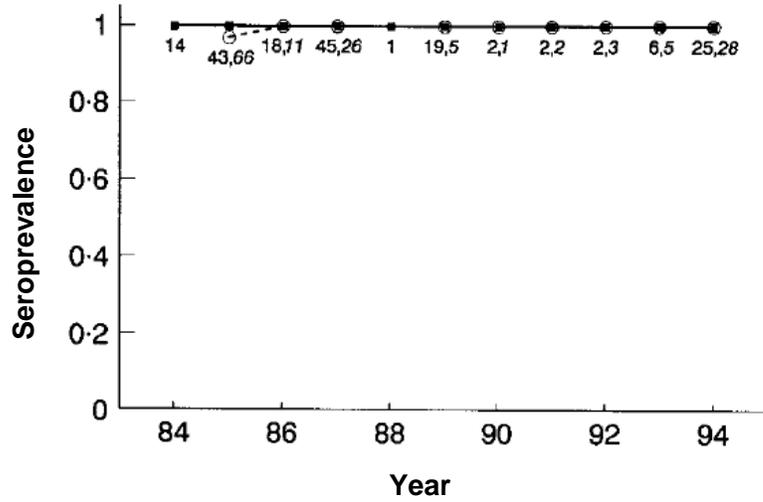


Figure 3. Annual seroprevalence rates in the Serengeti for feline herpesvirus for young lions (dotted line and circles) and for adults (solid lines and squares), with respective sample sizes (with numbers of immatures listed second, in italics) (From Packer et al. 1999).

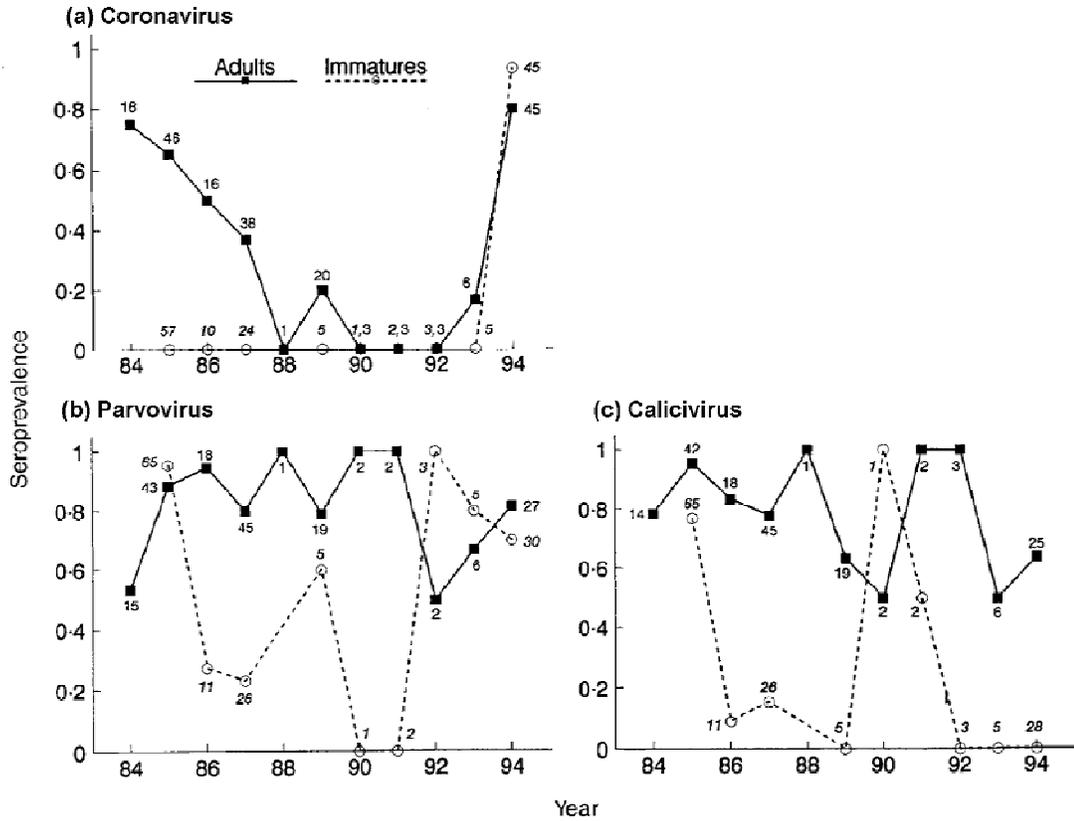


Figure 4. Annual seroprevalence rates in the Serengeti for (a) coronavirus (b) parvovirus and (c) calicivirus for young lions (dotted line and circles) and for adults (solid lines and squares) with sample sizes represented as numbers in the graph (From Packer et al. 1999).

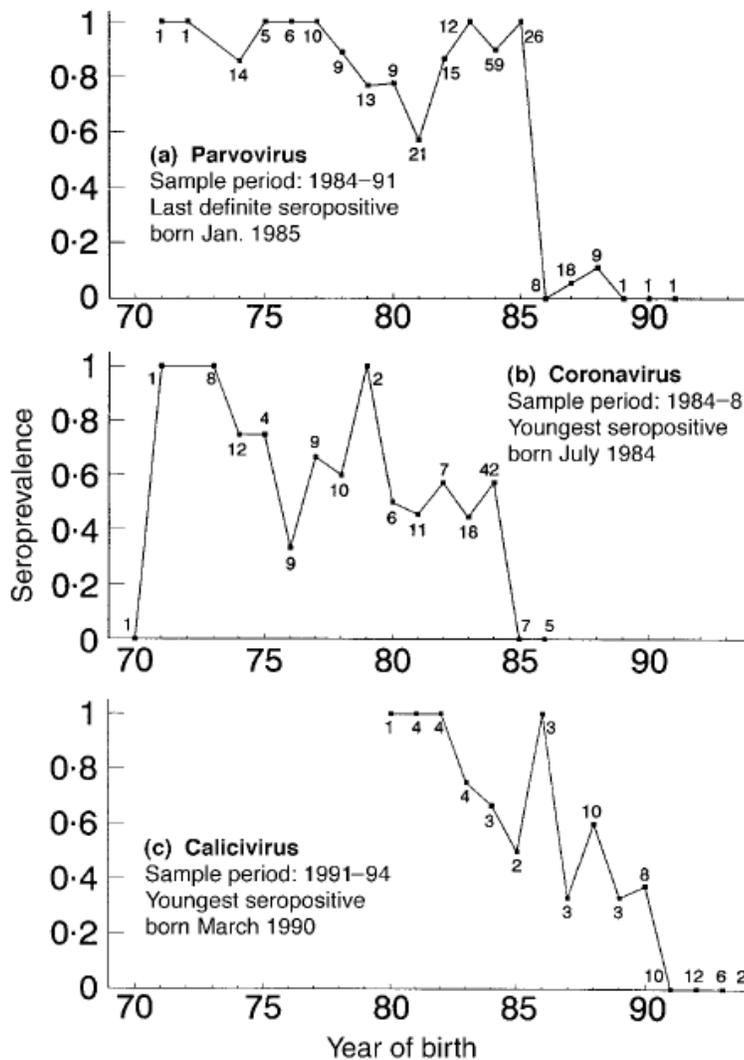


Figure 5. Representative seroprevalence curves with sample sizes indicated inside the graph. (a) Seroprevalence data for parvovirus is plotted according to year of birth for lions sampled between 1984 and 1991. Prevalence +/- 4 years of 1985 varied significantly. (b) For lions sampled between 1984 and 1988, coronavirus prevalence varies significantly for animals born +/- 4 years of 1984. (c) Seroprevalence for calicivirus for lions sampled between 1991 and 1994. Prevalence varies significantly for Animals born within +/- 4 years of 1990 (From Packer et al. 1999).

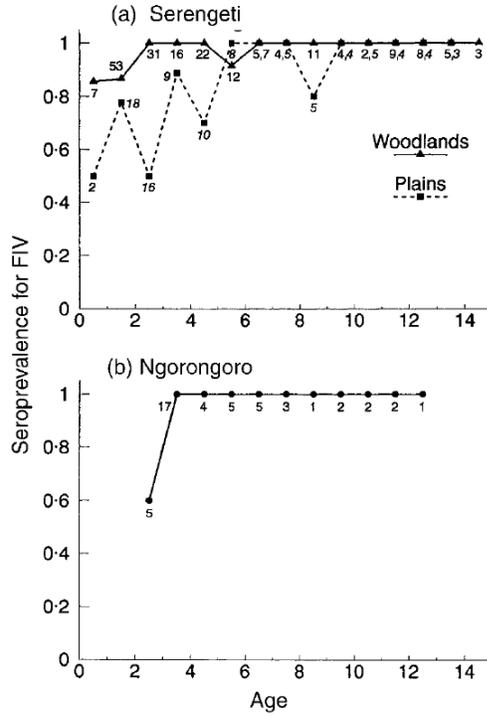


Figure 6. Age-prevalence curves for FIV averaged across 1984-1994 in (a) Serengeti woodlands and plains, and (b) Ngorongoro Crater. Yearly sample size is indicated beside each point the figure. Lions in the Serengeti plains show a lower rate of infection than the other two habitats ($T = -3.54$, $P < 0.001$), even when controlling for age in a multivariate analysis (effect of age: $T = 5.94$, $P < 0.001$; effect of Serengeti plains: $T = -3.98$, $P < 0.001$) (From Packer *et al*, 1999).

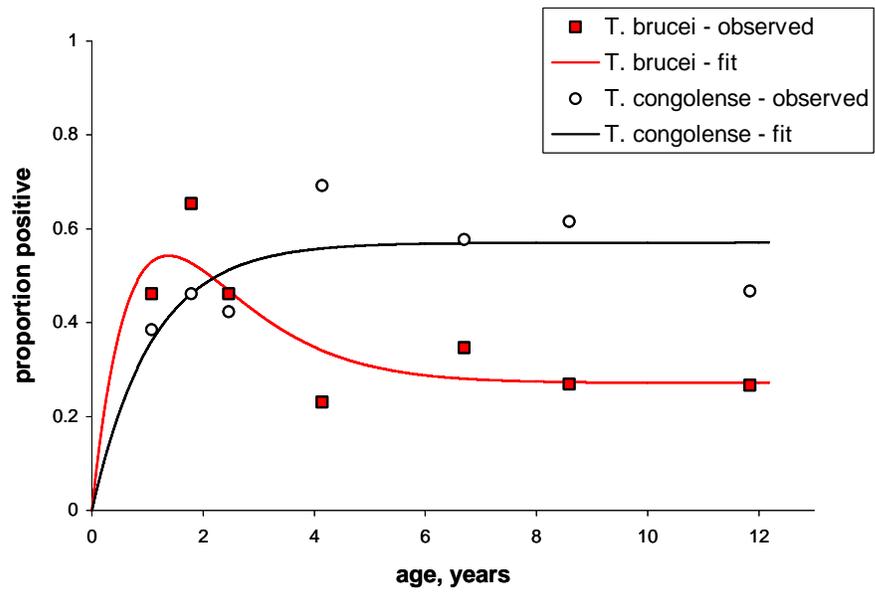
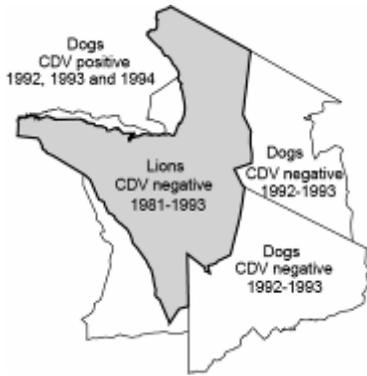
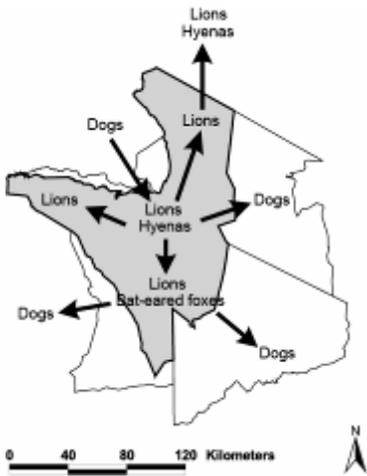


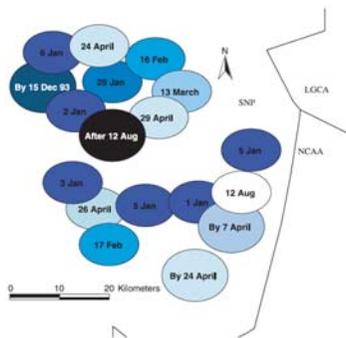
Figure 7. Model fitted to *T. brucei* and *T. congolense* age-prevalence curves in Serengeti lions. (For detailed description of model see Welburn *et al.* 2008.)



a.



b.



c.

Figure 8. Spatiotemporal spread of CDV. (a) Status of CDV before the 1994 epidemic in Serengeti lions, (b) its spread during the epidemic as reconstructed using serological or viral evidence (From Cleaveland *et al.* 2008), and (c) its spread among the lion study

population. Each oval represents a lion pride. The time course was determined by either the date of first observed death in a pride or date of sampling for the first seropositive individual in the pride. Prides infected early in the epidemic are colored dark, those infected in the epidemic grade through to white. One pride remained uninfected (black) (From Craft *et al.* 2008).

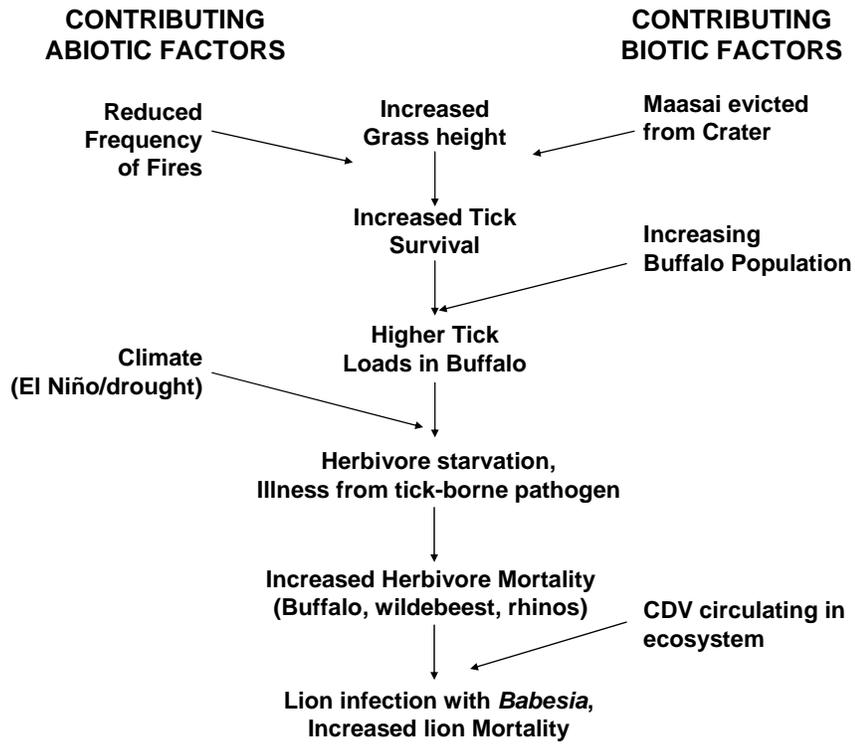


Figure 9. Abiotic and biotic factors in the Ngorongoro Crater which led to mortality in Crater lions (Fyumagwa *et al.* 2007, Munson *et al.* 2008).

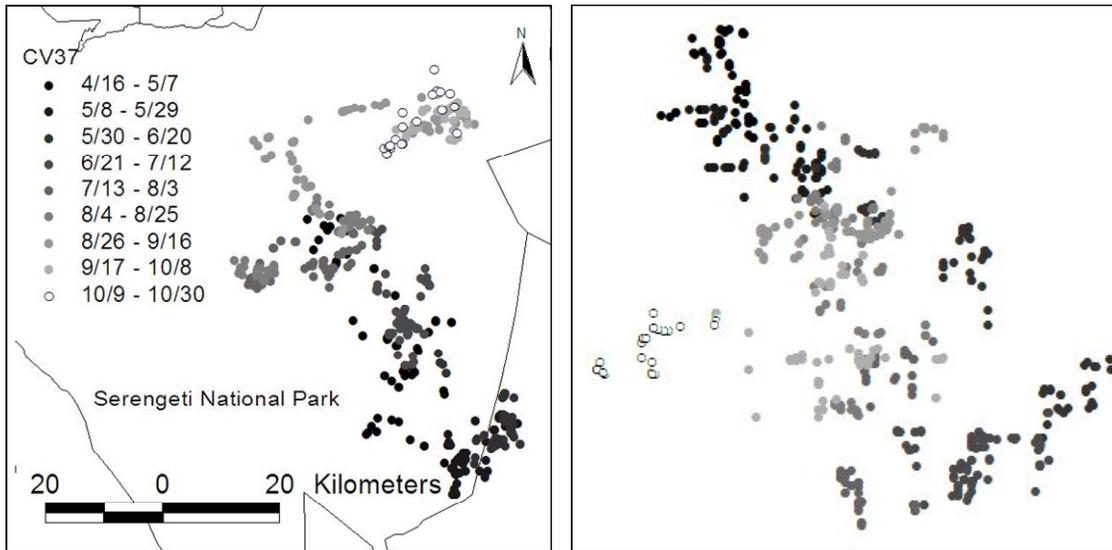


Figure 10. Movement patterns of a Serengeti nomadic lion in 2006 (left) where spatiotemporal locations are represented by shades of gray (dark are early locations whereas white are late locations and the month/day of locations are indicated in the legend) versus simulated nomad (right) with grey shades representing the same temporal scale (6 months). The time steps on the simulated nomad exactly mirror the time-steps on the actual (From Craft et al, in prep).

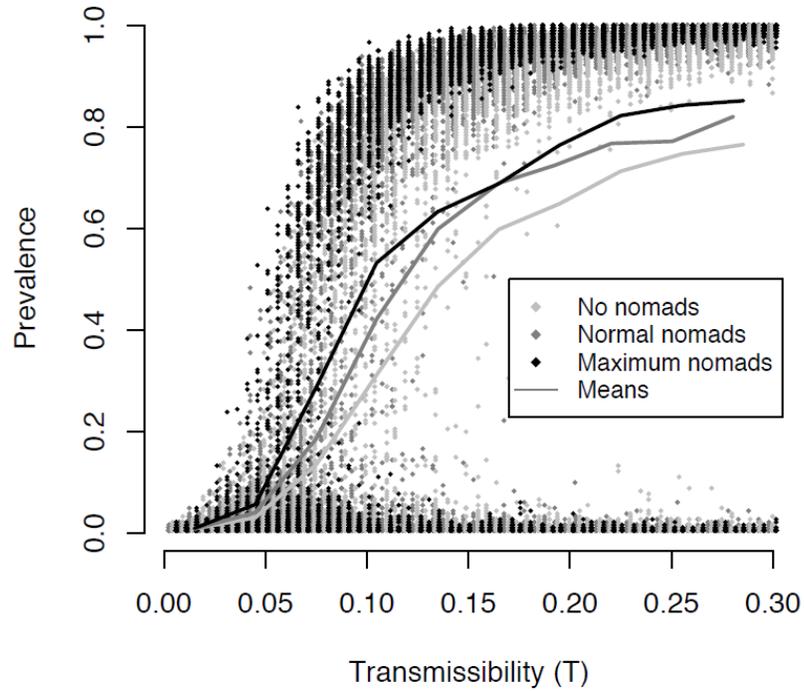


Figure 11. Prevalence across a range of transmissibilities for simulations with realistic movement patterns of nomads, “normal nomads,” and with maximum nomad migration and no nomads in the simulation. The mean for prevalence at each transmissibility was plotted for the overall model ecosystem (180 prides, solid lines) (From Craft et al, in prep).

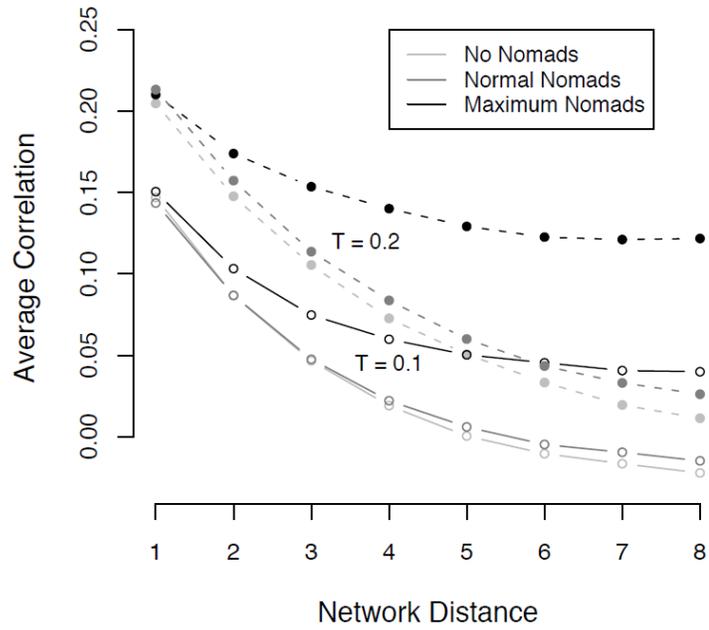


Figure 12. Network correlograms for simulated epidemics. In simulated epidemics, the average correlation in the timing of infectious periods between randomly chosen prides decreases with increasing network distance. This is plotted for transmissibility values of $T=0.1$ and $T=0.2$. Dashed lines, correlation when nomad movement is increased, and when nomads are removed (From Craft et al, in prep).

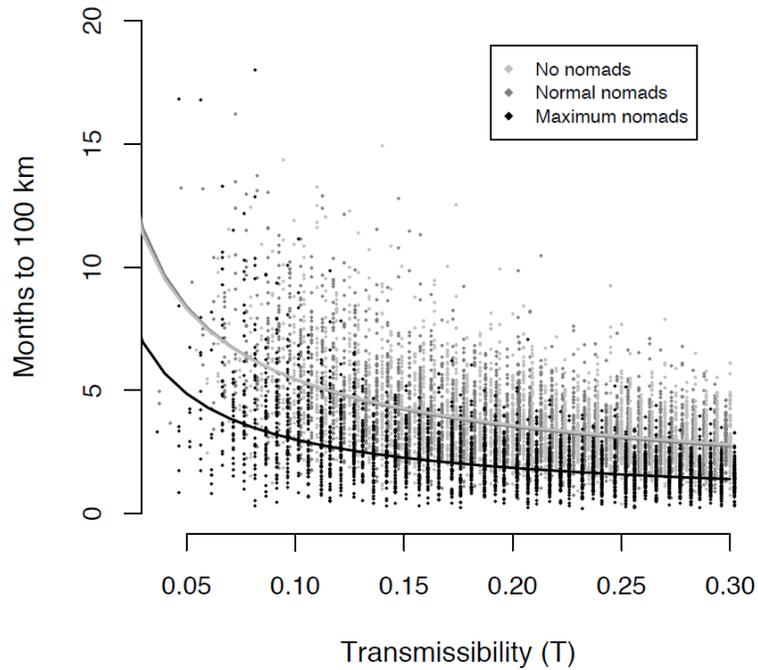


Figure 13. Velocity across a range of transmissibilities. Each point represents the time until the disease reached 100 km from the first infected pride for a single simulated epidemic starting at a randomly chosen pride in the subset. The lines show the least squares linear regression on log-log transformed values. The no nomads and normal nomads lines are on top of each other, while the maximum nomad line is below (From Craft et al, in prep).

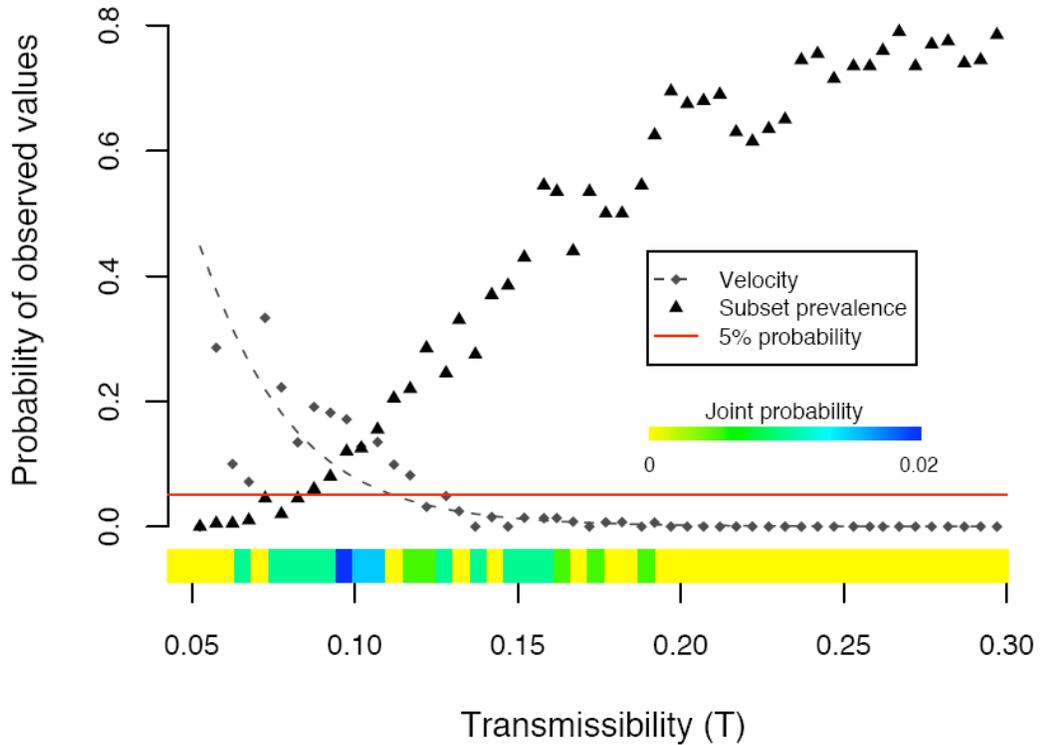


Figure 14. Probability of observed epidemic values across a range of transmissibilities. The probability of the observed velocity is calculated as the fraction of simulations that took at least 35 weeks to reach 100km. The probability of the observed prevalence is calculated as the fraction of simulations that infected at least 17 of the 18 prides in the subset. The red line at probability 0.05 indicates that there is a very limited range of transmissibility at which both patterns have at least a 5% of occurring. The joint probability is calculated as the fraction of simulations that exhibited both the observed velocity and prevalence (From Craft et al, in prep).

CHAPTER 2

Networks and nomads: Epidemiological structure and disease dynamics of a lion population[†]

Abstract

We estimated the epidemiological network structure of an African lion population using long-term data from the Serengeti Lion Project. We found that the lion population is a mix of local pride-to-pride contacts (driven by territory adjacencies) and transient nomad-to-pride contacts (driven by gamma variance process). When we introduced canine distemper virus (CDV) into the network, emulating a fatal 1994 outbreak, we found that although nomads are numerous, travel long distances, and are likely candidates to be considered “superconnectors” (connecting distant parts of a network), their impacts on CDV disease dynamics were surprisingly low. In our model, the inclusion of nomads slightly increased disease prevalence, but did not influence the velocity (rate of spread) or the pattern of spatial spread (correlations across distance). However, when the nomad movement rate increased, it changed disease dynamics by (a) increasing prevalence, (b) changing the spatial spread of the disease, and (c) increasing velocity. For diseases with relatively short infectious periods, like CDV, transients only slightly increase the already dense local pride-pride contact patterns and thus do not play pivotal epidemiological roles.

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Introduction

Canine distemper virus (CDV) swept through the Serengeti ecosystem in 1993-4, killing one-third of the well-studied lion population (Roelke-Parker *et al.* 1996). The virus was first detected in the Serengeti Lion Project's study population in December 1993 (Roelke-Parker *et al.* 1996), concurrent with the yearly arrival of migratory herds and associated nomadic lions (Maddock 1979). These non-residential nomadic lions wander great distances through the ecosystem following the seasonal migratory herds of wildebeest, zebra, and gazelle, which is in contrast to the majority of lions that live in territorial prides (Schaller 1972). Nomadic lions were suspected to be responsible for the introduction of CDV into the study population in 1993 and for long-range jumps of disease during the epidemic (Roelke-Parker *et al.* 1996).

Nomadic lions could be considered a variation of a “superspreader:” a “superconnector.” Superspreaders are a small fraction of a population who are responsible for most transmission events through excessive contacts (Lloyd-Smith *et al.* 2005); supershedders typically infect more individuals than others through excessive shedding of a pathogen; and we propose that superconnectors connect distant parts of a network through their long-range movements, increasing the extent of a disease outbreak. Network models capture these types of heterogeneous contacts and reveal the underlying population structure and contact patterns among individuals in the population.

Despite the importance of host heterogeneity for human disease transmission, relatively little is known about the epidemiological structure of wildlife populations (Krause, Croft & James 2007, Wey *et al.* 2008), and hence the individuals, or groups of individuals, that are responsible for most transmissions. Unfortunately, contact patterns are exceptionally difficult to measure in wildlife populations, and only a few free-ranging wildlife study systems are data-rich enough to provide empirical information to parameterize a network model (Cross, Lloyd-Smith & Getz 2005).

In this paper we characterize the epidemiological network structure of an African lion population using detailed data from the Serengeti Lion Project. Serengeti lions (*Panthera leo*) have been studied continuously since the 1960's; information

exists on individual ranging patterns, relatedness, and birthdates (normally accurate to 1 month). Contact patterns with conspecifics and other species can also be inferred. We build a network model of the lion population from the time period immediately before the 1994 CDV epidemic. To test the hypothesis that nomads have the potential to act as superconnectors, seeding new parts of the pride-pride network via long-range movements, we introduce CDV into the network model and investigate how the presence of nomads affects disease spread among Serengeti lions by (1) removing nomads from the network and (2) increasing the nomad movement rate.

Materials & methods

Lions live in gregarious groups (prides) composed of 1 to 21 related females, their dependent offspring, and a residential coalition of 1-9 males. Prides are territorial and infrequently contact their neighbors (Packer, Lewis & Pusey 1992); inter-pride encounters can be deadly (Schaller 1972, McComb *et al.* 1993, Grinnell, Packer & Pusey 1995). When prides grow too large, young females split off and form a neighboring pride (Pusey & Packer 1987) and are more tolerant of their non-pride relatives (VanderWaal, Mosser & Packer in press). Coalitions of males can be resident in more than one pride (Bygott, Bertram & Hanby 1979) and distribute their time between their various prides (Schaller 1972). In contrast, nomads are lions that do not maintain a territory and move great distances though the ecosystem (Schaller 1972). Lions from these three different social groups occasionally interact during mating, territorial defense, and at kills. Intuitively, nomads can be seen as long-distance disease dispersers while shared males can be viewed as increasing the level of disease transmission between neighboring prides.

Estimating Lion Population Structure

To estimate pride demographic structure and contact patterns prior to the 1994 CDV outbreak, we analyzed two datasets from the Serengeti Lion Project's 42 years of observations. The first recorded all lion sightings from October 1985 - December 1987, totaling 12,121 individual lion sightings. These records included time, location and

names of all lions observed, and descriptions of any interactive behavior among the lions. We analyzed data from 1985-1987 because of a contemporary dataset gathered during 35 four-day continuous follows of pride females (Packer, Scheel & Pusey 1990, Scheel & Packer 1991). Secondly, the demographic dataset described all lions in the study area on December 31, 1992, the last date of two-year average territory locations unaffected by the CDV die-off (Mosser 2008). We used the 1992 data for estimating the demographics prior to the 1994 CDV epidemic, and the 1985-1987 data for estimating all other parameters.

For most model parameters (Table 1), we characterized the entire distribution of values rather than single summary statistics. Unless otherwise specified, we used maximum likelihood estimation (MLE) to fit the parameters for seven candidate distributions (Poisson, exponential, normal, log-normal, pure power law, truncated power law, gamma), and then applied the Akaike Information Criterion (AIC) to select the most appropriate distribution.

Demographics and spatial distribution of prides

Prior to the 1994 outbreak, 25 prides lived in the study area. Pride sizes were calculated as the number of females and cubs over 3 months old; pride sizes averaged 10.5 individuals and the distribution was best fit by a gamma distribution (Table 1: X_p , Fig. 1a). We extrapolated the densities of lions found in the study area to an area of the ecosystem with similar habitat and expected densities (Table 1: $A = 10,000 \text{ km}^2$) and estimated $N_p = 180$ prides in the ecosystem. We defined the territory of a pride as its estimated 70% kernel over a two-year period (Mosser 2008), estimated the distance between prides using Euclidean distances between territory centroids (conceptual center of mass, or the center of an irregular territory), and considered two prides to be adjacent if their territories overlapped, touched, or were not separated by another pride territory (Fig. 2a). To determine the probability of two prides being classified as adjacent, logistic regression analysis yielded the following model (Fisher's exact test; $p = 0.1184$)

$$\ln\left(\frac{P_{adj(AB)}}{1 - P_{adj(AB)}}\right) = 1.483 - 0.386 \cdot S_{AB}$$

where S_{AB} is the number of other prides in the intervening region between A and B (Table 1: M_{adj} , Fig. 3). In the model, prides are distributed in uniform random locations in a square region; the location of a pride is a single point representing its territory centroid; and pairs of prides are assigned to be adjacent to one another randomly according to the estimated adjacency model (M_{adj}) (Fig. 2b); and these adjacencies form the edges of the territory network. This produces distributions of numbers of adjacent prides that are statistically similar to those calculated from the 1985-87 lion-sighting data (Fig. 2c,d, Fisher's exact test; $p = 0.2368$).

Pride-to-pride contacts

Lion prides are fission-fusion societies where lions associate in temporary subsets and frequently contact all members of their pride—with the exception of very small cubs (<3 months) which only associate with their mother (Schaller 1972, Packer, Pusey & Eberly 2001)—and were never observed to participate in any pride-to-pride contacts in the 1985-87 data set. We defined a potential CDV “contact” as being <1 meter from another individual or eating from the same food source immediately after another individual.

The rate at which prides contact other prides may depend on a number of factors. We performed logistic regression analysis to determine which of the following factors significantly relate to the likelihood that any two prides (A and B) will come in contact: (1) Euclidean distance between the centroids of the pride territories (x_{dist}), (2) distance between prides in the network of territory adjacencies (x_{net}), (3) the number of lions in pride A (x_{num}), and (4) whether or not the two prides had originated from the same pride within the last two years (x_{split}).

For any pair of prides A and B , the binary response variable for our logistic regression analysis was whether or not a sighting of A includes an interaction with B .

Each sighting of pride A thus yields whether or not it interacted with each of the other 24 prides. We analyzed a logistic regression model given by

$$\log\left[\frac{p}{1-p}\right] = \alpha + \beta_{\text{dist}}x_{\text{dist}} + \beta_{\text{net}}x_{\text{net}} + \beta_{\text{num}}x_{\text{num}} + \beta_{\text{split}}x_{\text{split}}$$

where the β terms are logistic regression coefficients and α is the intercept. This relates the interaction probability for a pair of prides (p) with the factors listed above. Only network distance and recent origin (or split) had significantly non-zero coefficients ($p < 0.0001$ and $p = 0.0019$, respectively). These were also the only two significant effects according to effect likelihood ratio tests that compare the full model to the model missing one of the independent variables ($p < 0.0001$ and $p = 0.0078$, respectively).

We therefore estimated the parameters in the (reduced) two-factor model given by

$$\ln\left(\frac{w_{c(A,B)}}{1-w_{c(A,B)}}\right) = \alpha + \beta_d d_t(A,B) + \begin{cases} -\beta_s & \text{if recently split} \\ \beta_s & \text{otherwise} \end{cases}$$

where $w_{c(A,B)}$ is the weighting factor for the contact rate between A and B , and $d_t(A,B)$ is the territory network distance between the prides. Specifically, $w_{c(A,B)}$ is the predicted probability that pride A will contact pride B per daytime sighting of pride A , which have an estimated average duration of one hour. We estimated the fraction of adjacent pairs (Ψ) that split from one another during the two-year period 1985-1987, and used this value to randomly assign common ancestry to adjacent pairs in the model.

From the empirical data there were 36 pride-to-pride contacts per 1294 hours of observation, translating to $C_p = 4.55$ contacts per 2 weeks "instigated" per pride. When a pride instigates a contact, the other pride is selected using probabilities that are weighted by a logistic function of their territory network distance (M_{contact}).

We used data from a four-day continuous follow study of individual lionesses (Packer, Scheel & Pusey 1990, Scheel & Packer 1991) to ask whether day- and night-contact rates might differ. When we extracted interpride contact rates from the focal follow data set, we were unable to reject the hypothesis that night and day contact rates for individual lionesses were identical (directional Wilcoxon Sign-Rank, $p = 0.109$). We also used the focal follow data to make an independent estimate of average pride-to-pride contact rates. For each of the focal lionesses, we calculated the per hour rate of contacts with lions from other prides (mean = 0.0044 ± 0.0035). To compute a comparable quantity from the lion-sighting data used in our model, we assumed that all pride lions had an equal probability of participating in any given contact with another pride. For each pride we calculated the following estimate of the per lion contact rate:

$$\frac{\text{average lions per contact}}{\text{number of lions in pride}} \cdot \frac{\text{number of inter-pride contacts}}{\text{hour}}$$

Averaging over all prides, we calculated an average rate of 0.0059 ± 0.00329 , consistent with the focal-follow data.

Any given inter-pride contact will involve one or more lions from each pride. A simple linear regression analysis suggested that contact group sizes depended significantly on pride sizes ($p = 0.0048$). To correct for increasing variance in group size with increasing pride size, we transformed group size by $G' = \log(G + 1)$. To model contact group sizes for any given pride of size N , we then sample from a normal distribution with mean given by the resulting regression equation, $\mu_{G'} = 0.447 + 0.014 \cdot N$, and standard deviation given by the standard deviation of the (normally-distributed) residuals, $\sigma_{G'} = 0.232$ (Fig. 4). The average number of pride lions participating in inter-pride interactions (G) is 3.65.

Territorial males

Territorial males live in coalitions that associate with one or more prides. In 1991-1992, 88.2% of territorial coalitions associated with a single pride and the remaining 11.8%

associated with two prides (η). For the two-pride coalitions, we estimated the rate of pride switching using data from a radio-collared territorial male who switched prides approximately once every 4.0 days (Kissui & Sorensina, unpublished data). If a territorial coalition l is associated with $\eta_l > 1$ prides, it will be assigned to one of its prides and migrate from one (i) to another (j) with probability

$$\mu_{ij} = (1 - \exp(-\zeta h)) / (\eta_l - 1)$$

where h is a small time-step and ζ is the territorial male migration rate.

Nomadic lions

Groups of nomadic lions roam through the ecosystem, occasionally contacting pride lions. We estimate $N_N = 180$ groups of nomads with a mean group size of 1.5 individuals where the group size (X_N) follows an approximately log-normal distribution (Fig. 1b).

The location of a radio-collared nomad was recorded 2-5 times a day for 9 months (Fig. 5a). At small timescales, displacements showed high variance due to occasional long-range movements, while at long timescales, displacements resembled Brownian motion (random movements). We thus model nomad movement as a variance gamma process (Madan, Carr & Chang 1998, Glasserman 2004), such that every displacement is a sum of two gamma random variables (one corresponding to longitudinal movement and the other corresponding to latitudinal movement) with scale proportional to time. This is a type of Lévy random walk. Specifically, nomads approximately move according to the following pair of equations

$$\begin{aligned} x(t+h) &= x(t) + \delta_{x,R}(h) - \delta_{x,L}(h) \\ y(t+h) &= y(t) + \delta_{x,U}(h) - \delta_{x,D}(h) \end{aligned}$$

where $x(t)$ and $y(t)$ are the horizontal and vertical positions of a nomad at time t ; $\delta_{x,R}(h)$ and $\delta_{x,L}(h)$ are the right and left displacements in an interval h and are random variables from identical gamma distributions, Γ_x ; $\delta_{x,U}(h)$ and $\delta_{x,D}(h)$ are the upwards and downwards displacements in an interval h and are random variables from identical gamma distributions, Γ_y . The parameters for these two gamma distributions are given in Table 1: M_{nomad} . An example of the movement of a simulated nomad is shown in Figure 5b. When we compared the x and y-displacements from the model nomads versus the actual nomad, the distributions were not statistically different (Cramer Von Mises Test, x-displacement: $p = 0.0732$, y-displacement: $p = 0.25$).

Each group of nomads is initially assigned to the territory of a randomly selected pride, and at any point in time thereafter, resides in or around the territory of exactly one pride. In any small time step h , the nomads will migrate from the territory of the current pride (i) to that of another pride (j) with probability given by

$$Z_{ij} = \left(1 - \left(1 - \left(F(d_{ij} + \alpha/2) - F(d_{ij} - \alpha/2) \right) \right)^h \right) / c_i$$

where $F(\)$ is the cumulative distribution function for displacement over a two-week period; d_{ij} is the distance between the centroids of territories i and j ; α is the average pride territory width ($\alpha = \sqrt{A/N_p}$); and c_i is a normalizer. Nomads are assumed to contact the local pride at a uniform rate approximating 7.136 contacts per two-week period (C_N).

Simulating disease spread

We model disease dynamics using a stochastic SEIR (susceptible-exposed-infectious-recovered) approach. Lions frequently contact all other lions in their pride, nomadic group or coalition, so we assume that any given pride, nomadic group, or resident-male coalition moves through the four disease classes as a unit, as in a Levins-type patch model (Levins 1969, Hanski & Gilpin 1997). A group is considered exposed when its first member becomes infected; the group transitions stochastically from exposed to

infectious at a rate of 1/7 per day and from infectious to recovered at a rate of 1/14 per day.

When an infected group (A) contacts a susceptible group (B), the probability of disease transmission is a function of the number of individuals involved in the interaction and a per-contact transmissibility parameter (T), given by

$$\tau_{AB} = \sum_{j,k} p_j q_k \left(1 - (1-T)^{j \cdot k}\right)$$

where p_j and q_k are the probabilities that the group sizes from A and B are j and k , respectively. This assumes that every lion in one group encounters every lion in the other group (remembering that a “group” of pride members does not necessarily include all of the pride, as described by (G)). When a susceptible coalition of territorial males resides with an infected pride, the coalition is immediately infected; when an infected coalition of territorial males switches to a susceptible pride, it immediately infects the second pride.

Unless stated otherwise, the analysis is based on 200 simulated epidemics at 60 transmissibility values (T) between 0.0 and 0.3. For each run, a new lion population network was generated randomly, parameters were set to the values given in the *estimated quantities* column of Table 1, and the first pride infected was chosen at random.

Results

We conducted three experiments to determine the impact of transient lions on disease dynamics: simulations with no nomads, nomads with realistic movement rates, and nomads with exceedingly high movement rates (specifically, random movement throughout the whole network). We found that the observed level of nomadism has a surprisingly low impact on canine distemper virus transmission. For example, for extensive CDV outbreaks with 95% prevalence, nomads only accounted for 10% of all transmission events. The vast majority of transmissions were pride-to-pride (immediate

neighbors, 53.1%; second degree neighbors, 27.8%, and third degree neighbors 8.3%), while less than 1% of transmissions were due to more distant pride-pride transmission and shared males. The same trends were observed for outbreaks where only 50% of prides were infected (Fig. 6). In contrast, when nomad movement rate was at a maximum, nomads were responsible for 20.2% of transmission events in large-scale outbreaks (Fig. 6).

We then examined the prevalence of CDV across a full range of transmissibilities. In general, as CDV transmissibility increased, so did overall prevalence; the pattern was bimodal with most simulations infecting few prides at low transmissibilities ($T < 0.10$), while most prides were infected at larger transmissibilities ($T > 0.10$). Accelerating the movement rate of nomads only slightly increased mean CDV prevalence whereas removing nomads from the simulations slightly decreased mean prevalence among prides (Fig. 7). These trends were consistent across all values of T .

Because the spatial spread of CDV was driven by neighbor-to-neighbor pride transmission, the pattern of spatial spread of CDV was highly correlated at close network distances, and less correlated at farther distances, manifesting in a wave-like spread (Fig. 8). Spatial spread was consistently higher correlated at larger transmissibilities ($T=0.20$ vs. $T = 0.10$). Nomads did not drive the dynamics of spatial spread, which remained essentially unchanged when we removed nomads from the “normal” simulations. However, increasing the nomad movement rate increased the correlation at longer network distances with more prides getting infected at roughly the same time across further distances.

CDV traveled faster at higher transmissibilities than at lower transmissibilities. Because there was no difference in the velocity of the epidemic when nomads were removed from the simulations, nomads were again unlikely to have driven the spatial spread (Fig. 9).

Discussion

The Serengeti lion population is a mix of a static pride-to-pride contacts and transient nomad-to-pride contacts. We developed innovative techniques in our network model, specifically constructing: (1) a statistical model for determining whether two prides are "adjacent" as a function of the geometric organization of the entire population, (2) a statistical model for contact rates between two prides as a function of distance within the spatial adjacency networks, also accounting for recently split prides and shared males, (3) a simulated population with variable pride and group sizes, and (4) a pattern of nomad movement as a type of Lévy process (gamma variance model), which produced large jumps over short time-scales, but a relatively homogeneous distribution of jumps over longer time-scales.

Because nomads are numerous, travel long distances, and are likely superconnectors, we were surprised to find that nomads moving at realistic rates have a low impact on canine distemper virus dynamics. However, for diseases with relatively short infectious periods, like the two weeks of CDV, nomads do not appear to be superconnectors because although nomads constitute half of all epidemiological groups (180 groups of nomads vs. 180 prides), they are only responsible for 10% of all transmissions. Because disease transmission is a function of both group size and the transmissibility parameter, and the average group size for nomads is 1.5 whereas the average pride size is 3.7, the differences in group size could possibly explain for the low rate of nomad disease transmission. Also, when the simulated nomad movement rate was increased, prevalence, spatial spread, and velocity of the outbreak all increased, suggesting that nomadic lions are insufficiently mobile to produce significant outcomes on CDV dynamics.

Network models can be used to identify high-risk populations to target for disease control. If nomads had been identified as superconnectors, theoretically they would be the social class of lions on which to focus disease-control efforts. However, the results of our network model indicate that because pride-pride transmission is driving disease dynamics, control efforts for short-lived pathogens should focus on pride members.

Table 1. (a) Lion demographics, (b) contact parameters, (c) epidemiological parameters (CDV)

Demographic parameters	Estimated quantities	Distributions [‡]	Reference
A : area of ecosystem	10,000 km ²	N/A	(Packer 1990)
N_p : number of prides in ecosystem	180	$U(150,200)^*$	(Packer 1990)
X_p : pride sizes (number of females and cubs over three months old)	X_p : Gamma(k, θ) with $\theta = 4.707$, $k = 2.226$ Mean pride size = 10.48	θ : $N(4.707, 1.243)$ k : $N(2.226, 0.636)$	Pride Sheets (PS) 91-92
η : fraction of prides that share territorial males with one other pride	0.882	η : $N(0.882, 0.078)$	PS 92
ζ : rate at which territorial male coalitions switch prides	0.25 switches/day	ζ : $N(0.25, 0.070)$	Kissui, unpublished
M_{adj} : territory adjacency model	$\ln\left(\frac{P_{adj(AB)}}{1 - P_{adj(AB)}}\right) = 1.483 - 0.386 s_{AB}$ (s_{AB} = the number of prides located in the joint radius of A and B) Mean number adjacent prides = 7.36	intercept $\sim N(1.483, 0.22)$ slope $\sim N(-0.386, 0.041)$	PS 91-92
Ψ : proportion of adjacent prides recently split from a common pride	0.063	$\Psi \sim N(0.063, 0.021)$	PS 85-87
N_N : # nomads	180	$U(150-200)^*$	PS 92
X_N : nomad group sizes	X_N : Log-normal(μ, σ) with $\mu = 0.292$, $\sigma = 0.446$ Mean group size = 1.51	$\mu \sim N(0.292, 0.065)$ $\sigma \sim N(0.446, 0.046)$	PS 92
M_{nomad} : nomad movement model Horizontal (x) and vertical (y) displacements per day are given by gamma distributions	Disp _x : Gamma _x (k_x, θ_x) with $k_x = 0.382$, $\theta_x = 2.85$ Disp _y : Gamma _y (k_y, θ_y) with $k_y = 0.714$, $\theta_y = 1.743$	$k_x \sim N(0.382, 0.029)$ $\theta_x \sim N(2.85, 0.02)$ $k_y \sim N(0.714, 0.029)$ $\theta_y \sim N(1.743, 0.019)$	M.C unpublished

[‡] Confidence intervals marked with an asterisk (*) are best guesses made by M.C. and C.P.

Contact parameters			
C_p : average rate of pride-pride contacts per pride	4.55 contacts/two weeks	$C_p : N(4.55, 0.573)$	PS 85-87
M_{contact} : contact weighting model	$\ln\left(\frac{w_{c(A,B)}}{1-w_{c(A,B)}}\right) = \alpha + \beta_d d_t(A,B) + \begin{cases} -\beta_s & \text{if recently split} \\ \beta_s & \text{otherwise} \end{cases}$ $w_{c(A,B)}$ is the weighting factor for the contact rate between A and B and $d_t(A,B)$ is the territory distance between the prides.		PS 85-87
α : intercept	3.265	$\alpha : N(3.265, 0.371)$	PS 85-87
β_d : network distance coefficient	1.698	$\beta_d : N(1.698, 0.264)$	PS 85-87
β_s : recent split coefficients	0.696	$\beta_s : N(0.696, 0.220)$	PS 85-87
C_N : average rate of pride-nomad coalition contacts per pride	7.136 contacts/two weeks	$N(7.136, 1.018)$	PS 85-87
G : pride group size during contact $G' = \log(G + 1)$	$G' \sim N(\mu_{G'}, \sigma_{G'})$ with $\mu_{G'} = 0.447 + 0.014 \cdot X_p$, $\sigma_{G'} = 0.232$ Mean group size = 3.65	$\mu_{G'}$ intercept $\sim N(0.447, 0.004)$ $\mu_{G'}$ slope $\sim N(0.014, 0.004)$ $\sigma \sim N(0.232, 0.022)$	PS 85-87
Epidemiological Parameters			
ε : incubation period (days)	ε : Exponential(λ) with $\lambda = 1/7$	N/A	(Appel 1987)
l : infectious period (days)	l : Exponential(λ) with $\lambda = 1/14$	N/A	(Greene & Appel 2006)

FIGURES

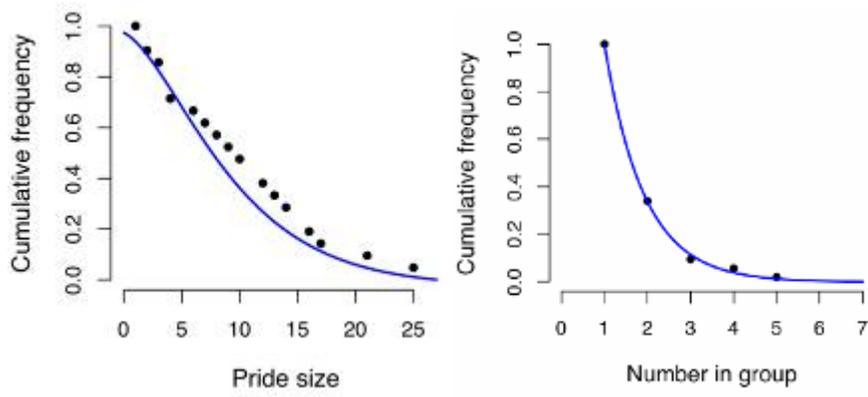


Figure 1. Distributions of (a) pride size and (b) nomadic group size.

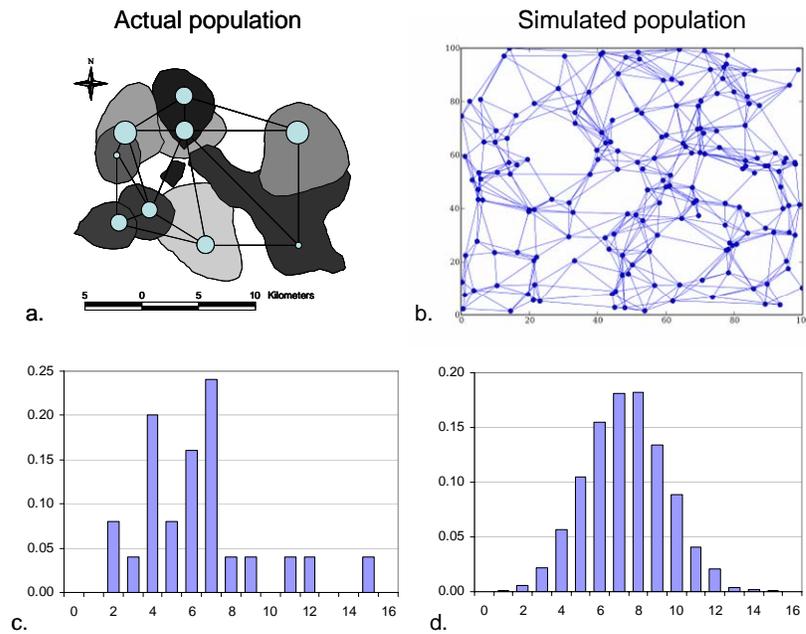


Figure 2. Comparison of actual (a,c) and simulated (b,d) lion populations. (a) Irregular shapes represent 70% kernel lion territories; nodes represent pride centroids where larger nodes represent larger pride sizes; edges represent adjacencies between prides. (b) simulated lion population with nodes (prides) and edges (adjacencies). Number of adjacent prides for the (c) SLP study population and (d) simulated lion population.

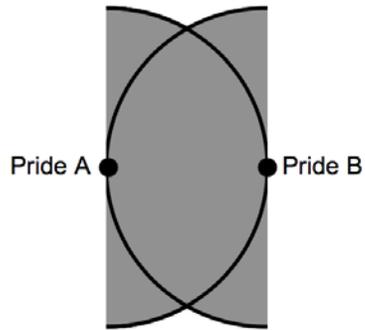


Figure 3. Adjacency Model. S_{AB} is the number of other prides in the intervening region between A and B , shown in gray.

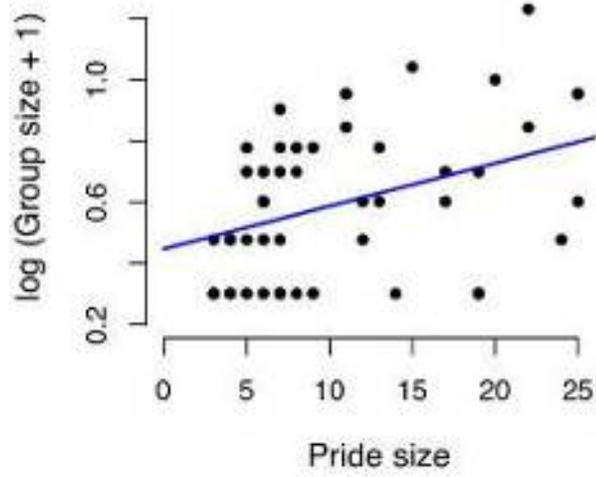


Figure 4. Total pride size vs. group size participating in an interaction.

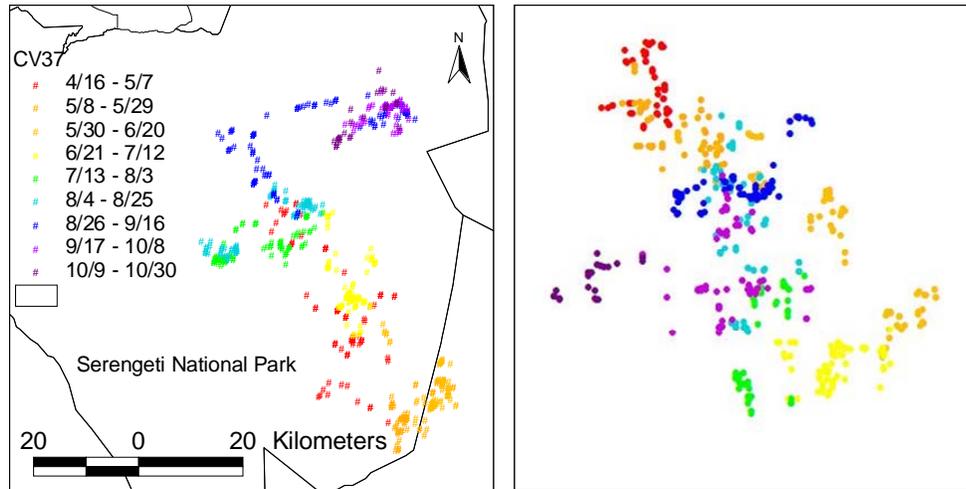


Figure 5. Movement patterns of a Serengeti nomadic lion (left) where spatiotemporal locations are represented by colors of the rainbow (red are early locations whereas purple are late locations versus simulated nomad (right) with colors representing the same temporal scale (6 months). The time steps on the simulated nomad exactly mirror the time-steps on the actual.

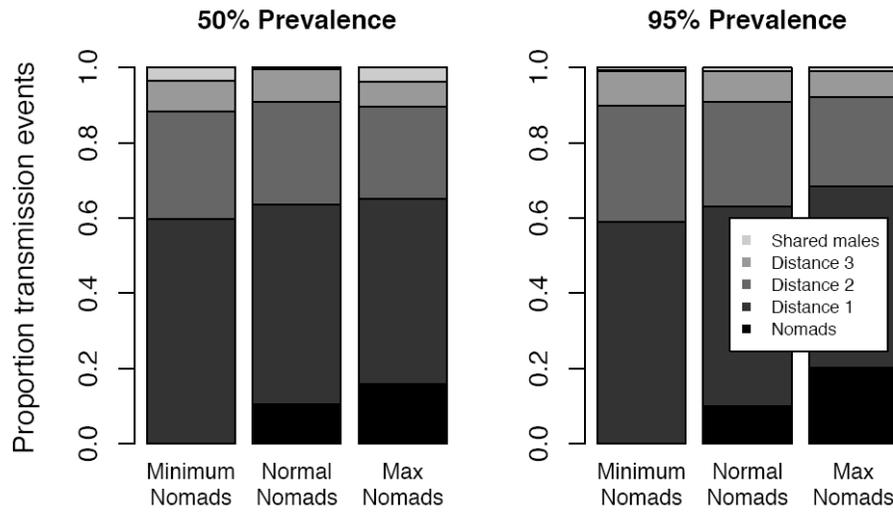


Figure 6. Proportion of transmission events for scenarios of no nomads (“minimum nomads”), normal nomad movement rate, and maximum nomad movement rate at 50% and 95% pride prevalence for the following groups of lions: nomads, prides at network distance 1, 2, and 3, and for shared males (and prides at network distances ≥ 4).

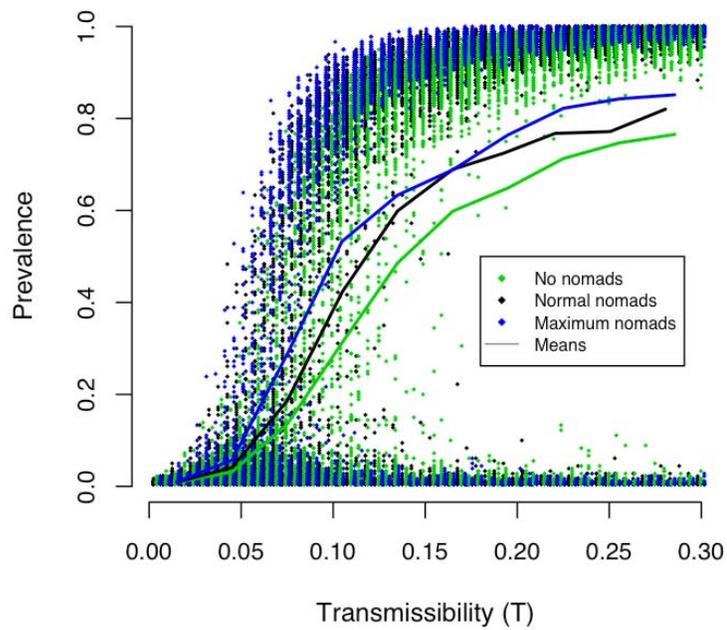


Figure 7. Prevalence of CDV in prides across a range of transmissibilities for simulations with realistic movement patterns of nomads (“normal nomads”) and with maximum nomad movement and no nomads in the simulation.

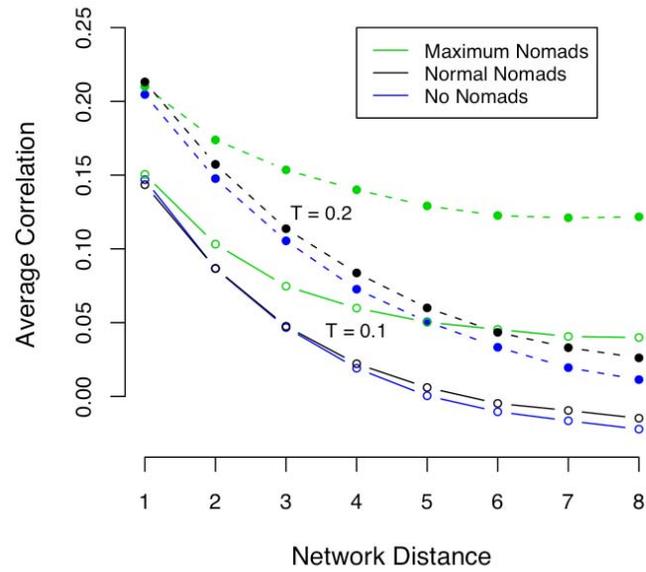


Figure 8. Network distance vs. average correlation at two transmissibility values. Green lines, correlation when nomad movement is increased, and blue lines, when nomads are removed. The error bars overlap considerably so were not shown.

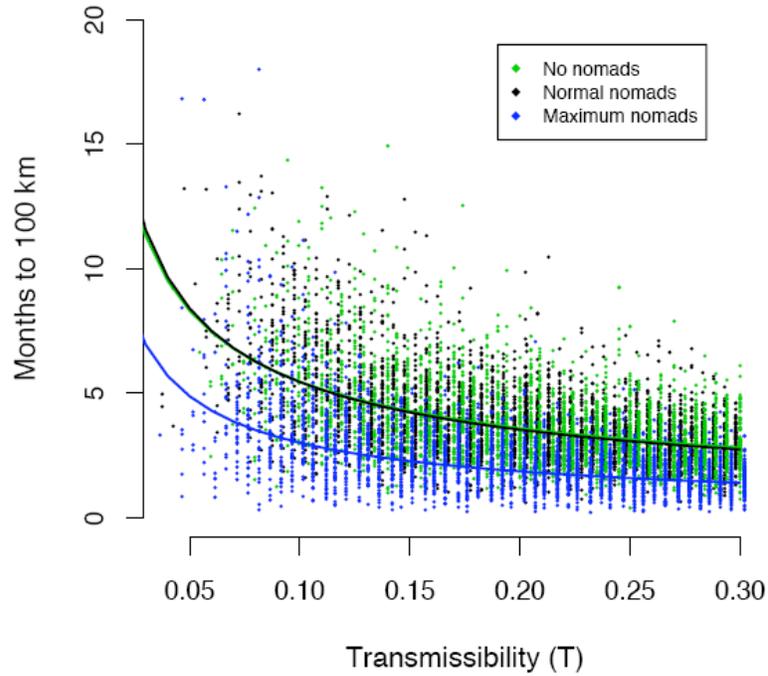


Figure 9. Velocity across a range of transmissibility values. Each point represents the time until the disease reached 100 km from the first infected pride for a single simulated epidemic starting at a randomly chosen pride in the subset. The black line shows the least squares linear regression on log-log transformed values, while the blue lines are the minimum and maximum nomad values.

CHAPTER 3

Distinguishing epidemic waves from disease spillover in a wildlife population[§]

Summary

Serengeti lions frequently experience viral outbreaks. In 1994, one-third of Serengeti lions died from canine distemper virus (CDV). Based on the limited epidemiological data available from this period, it has been unclear whether the 1994 outbreak was propagated by lion-to-lion transmission alone or involved multiple introductions from other sympatric carnivore species. More broadly, we do not know whether contacts between lions allow any pathogen with a relatively short infectious period to *percolate* through the population (that is, reach epidemic proportions). We built one of the most realistic contact network models for a wildlife population to date based on detailed behavioral and movement data from a long-term lion study population. The model allowed us to identify previously unrecognized biases in the sparse data from the 1994 outbreak and develop methods for judiciously inferring disease dynamics from typical wildlife samples. Our analysis of the model in light of the 1994 outbreak data strongly suggests that, although lions are sufficiently well-connected to sustain epidemics of CDV-like diseases, the 1994 epidemic was fueled by multiple spillovers from other carnivore species, such as jackals and hyenas.

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Introduction

Effective management of wildlife diseases depends on reliable information about transmission patterns, and, at the very least, knowing which species participate in transmission as maintenance, and non-maintenance hosts (Cleaveland *et al.* 2007). Maintenance populations steadily maintain disease for long periods of time and can serve as disease reservoirs (Haydon *et al.* 2002). They typically exceed a critical community size (CCS) in which a pathogen can persist indefinitely (Bartlett 1960). Non-maintenance populations can experience transient outbreaks, which are either large epidemics that reach a significant fraction of hosts or small outbreaks that die out after only a few infections. There are two distinct classes of non-maintenance host populations: percolating populations can (but do not always) sustain large epidemics while non-percolating populations cannot (Newman 2002, Meyers *et al.* 2005, Bansal, Grenfell & Meyers 2007, Davis *et al.* 2008). Whether or not a non-maintenance population can sustain an epidemic on its own depends, in part, on contact patterns among hosts. Populations with ample opportunities for pathogen transmission will lie above the *epidemic threshold* where large epidemics are possible, while more sparsely connected populations will lie below the epidemic threshold where outbreaks rapidly fizzle out.

Disease control strategies should prioritize maintenance hosts (Haydon *et al.* 2002). However, for direct intervention in non-maintenance populations, it is critical to determine whether or not the population is percolating or non-percolating. If a non-percolating population experiences repeated introductions of diseases from sympatric populations, it may experience a series of small outbreaks that together take a large toll on the population. Multiple spillover outbreaks like these may superficially resemble a single epidemic wave; however, the optimal control strategies for these two scenarios are quite different. In the spillover case, control measures should focus almost exclusively on preventing new introductions of disease, whereas in the epidemic case, strategies should also target transmission within the host population. Incorrectly targeting interventions can waste precious resources and cause harm to wildlife [e.g.

extermination of Asian civets for SARS (Li *et al.* 2005) and UK badgers for bTB (Donnelly *et al.* 2006)].

Mathematical models have historically provided important insights into disease dynamics and management (Anderson & May 1991, Ferguson, Donnelly & Anderson 2001, Haydon, Laurenson & Sillero-Zubiri 2002, Keeling & Rohani 2008). Traditional disease models can, however, be misleading: mass-action models assume that populations are fully mixed, and lattice-based spatial models assume that all contacts are spatially proximate. Endangered species often live in groups and defend territories against conspecifics (e.g., lions in prides, wolves in packs), thus exhibiting population structure that is neither fully mixed nor geographically localized. Their populations show “community structure” (Cleaveland *et al.* 2008) in which the groups are highly intraconnected and more loosely interconnected based on complex movement and behavioral patterns. Epidemiological data corroborates that social groups are often the critical units for disease transmission in wildlife (Altizer *et al.* 2003).

Contact network models allow us to explicitly consider the epidemiological consequences of complex patterns of host connectivity and have demonstrated that contact heterogeneity can fundamentally influence disease dynamics (Keeling 2005, Meyers *et al.* 2005, Bansal, Pourbohloul & Meyers 2006, Ferrari *et al.* 2006). However, network modeling often suffers from a paucity of good data on contact patterns, particularly for non-human hosts. Very few studies of free-ranging wildlife provide adequate empirical information to parameterize a network model (Cross, Lloyd-Smith & Getz 2005); but the long-term data set of the Serengeti Lion Project (SLP), which include decades of daily observations of behavior and movement, is a unique exception (Packer *et al.* 2005).

We used the SLP data to infer the contact network structure of an African lion (*Panthera leo*) population and built one of the most detailed, biologically realistic epidemiological network models of a wildlife population to date [but see (Cross, Lloyd-Smith & Getz 2005)]. The model incorporates pride composition, movement of nomads (roaming lions), and contact rates between prides and nomads into a stochastic SEIR (susceptible-exposed-infectious-recovered) network framework. Disease-causing

contacts between lions from different groups are assumed to include chases, fights, mating, close proximity, and sequential and simultaneous feeding events. We then used this model to ask whether lions alone can sustain epidemics of contact-borne infectious diseases without repeated introductions from other species and, specifically, whether an observed 1994 canine distemper virus (CDV) epidemic could have been propagated exclusively by lion-to-lion transmission. The 1994 epidemic spread discontinuously through the study area, infected 17 of 18 study prides, and took 35 weeks to spread across the entire ecosystem (Roelke-Parker *et al.* 1996, Cleaveland *et al.* 2007, Craft *et al.* 2008). Lions, hyenas (*Crocuta crocuta*), bat-eared foxes (*Otocyon megalotis*) and domestic dogs (*Canis lupus familiaris*) were all infected with the same strain of CDV (Haas *et al.* 1996, Roelke-Parker *et al.* 1996, Carpenter *et al.* 1998), thus supporting the possibility of cross-species disease transmission. While some studies have argued that the lions experienced repeated introductions from other carnivore species (Cleaveland *et al.* 2008, Craft *et al.* 2008), Guiserix *et al.* (2007) have claimed that, once CDV was introduced into the lion population, the lions likely sustained the outbreak themselves without subsequent transmission events from other species.

In addressing the plausibility of lion-to-lion transmission, we tackled larger issues about extrapolating disease dynamics from a geographically restricted study area (Figure 1A) to a greater ecosystem. By taking samples from comparable areas or “subsets” of our model ecosystems (Figure 1B), we identified several unexpected discrepancies between sample data and ecosystem-wide disease dynamics, which are likely to arise in many wildlife disease field studies. In contrast to prior studies of the 1994 CDV outbreak (Guiserix *et al.* 2007), we analyzed the field data in light of these discrepancies.

Results

We built an epidemiological network model, based on contact patterns within a lion population estimated from detailed SLP data. The core of the model was a *territory network* in which prides were aggregated into single units (nodes), and edges were drawn between prides with adjacent territories, based on observed data. The *territory*

distance between any two prides was then defined as the shortest path connecting their respective nodes. Prides contacted each other as a function of territory distance, and nomads migrated as a type of variance gamma process (Madan, Carr & Chang 1998, Glasserman 2004), contacting prides in their vicinity according to empirical estimates. Prides moved through each SEIR class as a unit, with disease progression parameters taken from published estimates for domestic dogs. (A description of the model and parameters used can be found in the Materials and Methods section). Simulations were run across a range of transmissibility (T) values (the probability of disease transmission during a contact between a susceptible and infectious lion). We monitored disease spread throughout the entire population and within a subset of 18 prides (Figure 1B) resembling the study population (Figure 1A).

Edge effects

We use two network quantities to characterize the location of a pride in the overall network. The *degree* of a pride is the number of directly adjacent neighboring prides; and the *closeness centrality* of a pride is the reciprocal of the pride's average minimum path length to all other prides in the network, which intuitively correlates with the likelihood that disease will reach the pride from elsewhere in the ecosystem. In our model, the subset prides were biased toward the physical and network boundaries of the ecosystem, having lower average distance to the ecosystem boundary, degree, and closeness centrality than the population as a whole (Figure 2, horizontal boxplots). These differences are statistically significant (Wilcoxon signed rank test, $p < 10^{-16}$ for all three comparisons).

We investigated the relationship between these metrics and the probability that a pride (1) will become infected during an epidemic and (2) can spark a large-scale epidemic in an immunologically naïve population (Figure 2, dotted lines). Both of these epidemiological risks increase with distance to edge, degree, and centrality of a pride. Multivariate logistic regression indicates that while degree and closeness centrality correlate significantly with the probability that a pride becomes infected ($P < 0.001$;

Table S1), distance to edge does not. These patterns explain the lower disease burden in the subset when compared to the overall population (Figure 3B).

Small sample size

During the 1994 CDV epidemic, 17 of 18 prides (94%) in the 2000 km² SLP study area were infected. Based on the edge effect, we initially assumed that the overall prevalence in the ecosystem should have been greater than or equal to this value. Instead, the model subset was more likely to experience an outbreak with $\geq 94\%$ of prides infected than the overall population (Figure 3C). This discrepancy has a simple combinatoric explanation. Consider a simple model in which: (1) prides infected during an epidemic are randomly distributed throughout the ecosystem, and (2) subsets are random samples of 18 prides from the set of 180 prides. Then subset prevalences should follow a hypergeometric distribution with parameters $N = 180$, $m =$ number infected prides overall, and $n = 18$. This null model closely predicts the observed differences (Figure 3C, blue line), even though it ignores spatial clustering of disease and the contiguity of prides in the subset.

Spatial scale

In the model, CDV epidemics typically spread wavelike across the ecosystem. Specifically, the shorter the distance between prides in the territory network, the higher the correlation between their times of infection (Figure 4A). The wavelike pattern is more pronounced when measured by network distance rather than geographic distance (not shown). When viewed through the narrow lens of the subset, however, there is a lower correlation for directly adjacent prides and almost no correlation among more distant prides (Figure 4A).

To compare correlograms across transmissibility values, we calculated correlations for directly adjacent prides (a network distance of one) and the slope of the correlogram for network distances between one and three (Figure 4C and 4D). For outbreaks that originated in the subset (as observed in the 1994 epidemic), correlations between adjacent prides increased with transmissibility, but correlations were lower in

the subset than across the entire population. The rate at which the correlations declined with network distance was similar in the subset and population and relatively uniform across all transmissibility values. Thus Figure 4A (which is based on $T = 0.1725$) is representative of the spatio-temporal patterns observed across the entire range of transmissibilities, with little apparent correlation in the subset despite a wavelike spread overall.

When we plotted distance from the first infected pride (*pride zero*) against the time of infection during a typical simulation (Figure 4B), we observed relatively continuous expansion overall, but a discontinuous pattern within the subset fueled by repeated introduction from elsewhere. For outbreaks initiated within the subset that infected at least 17 of 18 prides, the probability of at least one reintroduction was 0.970 (SD=0.093); and the average number of subset prides with a $\geq 75\%$ chance of infection from outside the subset was 1.96 (SD=1.73). Thus the spatial pattern of infections within the subset generally appeared patchy in the midst of a wavelike epidemic.

Model versus data: Did lions sustain the 1994 outbreak themselves?

We compared the predictions of our model to three empirical observations: the discontinuous spatial spread within the study area, 94% prevalence within the study area, and the slow spread of the outbreak across the entire ecosystem. We also performed a full sensitivity analysis and found that the quantitative results were largely insensitive to uncertainty in the parameter values (Table 1, S3).

The model produced spatial patterns within the subset that were similar to the 1994 outbreak (Figures 4 and 5). Disease appeared in clusters separated from each other in time and space. Across the entire range of transmissibility values, there is a 10-20% chance that epidemics will appear at least as discontinuous as observed in 1994 (Figure 4E). These probabilities are highest for low values of transmissibility, where transmission between neighboring prides is rare and thus the time of infection for adjacent prides is relatively uncorrelated. The model also predicts outbreaks with the observed pride prevalence, especially at higher transmissibility values (Figure 3A), and

predicts the observed rate of geographical spread at lower transmissibility values (Figure 6).

Although each of these individual patterns has a reasonable probability of occurring in a lion-to-lion epidemic, it is highly unlikely that all three could occur simultaneously (Figure 7). The observed spatial spread and velocity are most likely to occur at low transmissibilities while the observed prevalence is most likely at higher transmissibilities. Only a minute fraction of simulations exhibited both the observed prevalence and velocity. The highest probability of observing both patterns is 0.02, occurring at approximately $T = 0.095$. We did not include the spatial analysis (Figure 4) in this comparison because patchy outbreaks correlate with low velocity and adding a spatial criterion would only reduce the joint probability further.

Since the model failed to identify a range of transmission values that could have plausibly produced an epidemic that was both as large and as slow as the observed 1994 outbreak, we conclude that the assumption of strict lion-to-lion transmission must be incorrect. Thus the actual transmission dynamics must have involved multiple introductions of disease to the lions from sympatric carnivore species.

Discussion

Are Serengeti lions a percolating population for CDV?

Serengeti lions likely experience outbreaks of CDV and other directly transmitted viral diseases with similar infectious periods, such as feline calicivirus and parvovirus, every four to twelve years (Packer *et al.* 1999). Our model suggests that this population of lions is sufficiently well-connected to sustain epidemics of CDV-like diseases on their own, that is, it is a percolating population for viruses with short infectious periods. Even moderately contagious diseases (with probability of transmission per contact $T \approx 0.13$), have at least a 5% chance of producing an epidemic that reaches 95% of all prides in the ecosystem (Figure 3C); and this probability increases rapidly with transmissibility. If CDV is at least moderately infectious in lions, as suggested for domestic and wild carnivores (Appel 1987), then our model suggests that it has the potential to sweep through the entire population.

The 1994 CDV outbreak, however, was unlikely to have been maintained by lions alone. Across the entire range of transmissibility values, a strictly lion-to-lion epidemic could not have been both as extensive and as slow-moving as observed in 1994. At low rates of transmissibility, disease can spread as slowly as in 1994 but not reach the observed prevalence; the reverse is true at high rates of transmissibility (Figure 7).

The most plausible explanation for this discrepancy is the absence of additional carnivore species from our model. Lions commonly contact hyenas and jackals during simultaneous or sequential feeding events (Cleaveland *et al.* 2008), and a single CDV variant was found to be circulating in lions, hyenas, bat-eared foxes, and domestic dogs during the 1994 outbreak (Haas *et al.* 1996, Roelke-Parker *et al.* 1996, Carpenter *et al.* 1998). Thus, there were repeated opportunities for CDV to be introduced into the lion population. Although this conclusion contradicts a recent analysis by Guiserix *et al.*, it is consistent with the genetic analysis and supported by observations of sick jackals and leopards at the time of the epidemic (Roelke-Parker *et al.* 1996) and the hypothesis that morbilliviruses require large critical community sizes (Bartlett 1960, Grenfell, Bjornstad & Kappey 2001). Our model suggests that lions were a “non-maintenance” population for this CDV epidemic and experienced transient chains of infection that “spilled over” from other species (Haydon *et al.* 2002, Fenton & Pedersen 2005).

Do disease dynamics scale?

Wildlife studies can be resource and time intensive, thus biologists regularly extrapolate from subsets of larger populations. Ecologists recognize that natural processes can vary considerably with the spatial scale of the observation (Tilman & Kareiva 1997, O'Neil & King 1998) and thus use multi-scale approaches to analyze complex ecological systems. Given the difficulty of observing wildlife disease outbreaks in real-time, disease ecologists are typically forced to mine sparse data without regard to sampling or scaling issues (examples include: (Williams *et al.* 1988, Woodroffe, Ginsberg & Macdonald 1997, Packer *et al.* 1999, Leendertz *et al.* 2004, Haydon *et al.* 2006)).

In this study we identified three potential sources of error that are relevant to wildlife disease ecology. The first is an edge effect, or more generally, non-random sampling with respect to the epidemiological structure of the population. Directly transmitted diseases spread primarily during contacts between neighbors or neighboring groups; and the pattern of such interactions gives rise to a contact network. The position of a group within the network, in conjunction with the overall network structure, determines its epidemiological risk (Figure 2). The contact network for Serengeti lions is highly spatial, such that contact rates are highly correlated with the number of nearby prides. Thus, prides located closest to the border of the Serengeti National Park have the fewest contacts, on average. For this reason, estimates based on samples taken from the outskirts of the park (like the SLP study area) would tend to underestimate the overall burden of disease in the Serengeti ecosystem. Note, however, that samples from a geographic boundary will not suffer from an edge effect if the population is sufficiently well mixed that contact rates are homogeneous throughout the ecosystem.

The frequency of an epidemic in the subset can also differ significantly from the overall population, simply because of variability associated with taking a small random sample from a large population. Just by chance, the sample proportion can deviate considerably from the population proportion. In the 1994 CDV epidemic, 94% of prides in the subset were infected. At relatively low transmissibilities ($T \sim 0.1$), almost no simulated epidemics reach an overall prevalence of 94%, yet a sizable fraction infect at least 94% of subset prides. This is predicted by a simple hypergeometric model in which we assume that the subset is a truly random sample from the population of 180 prides. For example, suppose an epidemic reaches 80% of all prides (144 of 180). A random sample of 18 prides will have roughly a 9% chance of having prevalence above the 94% threshold even though the population as a whole is well below the threshold. Thus at moderate transmissibilities, where few, if any, epidemics cross the 94% threshold, sampling variability alone can explain the higher vulnerability of the subset to large epidemics than the overall population.

The final complication arises when sampling from a smaller geographic scale than that of disease transmission. The SLP data from the 1994 CDV outbreak suggests

non-wavelike, erratic spread of disease through the study area which has been seen as evidence for repeated introduction from other species (Craft *et al.* 2008). Although we ultimately rejected the possibility that lions sustained the 1994 outbreak by themselves, it would have been incorrect to assume that the observed spatial spread necessarily implied a similar pattern across the entire ecosystem. While contacts primarily occur between neighboring groups, lion prides occasionally contact distant prides and migrating nomads, which reduces the correlation between distance and the timing of infection. When the probability of transmission is low, disease may initially reach only a few prides in a given area and later return to the same vicinity via longer-distance contacts. In a population with exclusively local contacts, dynamics at a small scale will become much more wavelike and more closely resemble the large-scale dynamics. On the other hand, completely mixed populations will lack scale-dependencies, because they lack spatial patterns altogether.

Materials & methods

Modeling Lion Population Structure

Lions live in gregarious groups (prides) composed of related females and their dependent offspring. Prides are territorial and infrequently contact their neighbors (Packer, Lewis & Pusey 1992); inter-pride encounters can be deadly (Schaller 1972, McComb *et al.* 1993, Grinnell, Packer & Pusey 1995). When prides grow too large, young females split off and form a neighboring pride (Pusey & Packer 1987) and are more tolerant of their non-pride relatives than lions from unrelated prides (VanderWaal, Mosser & Packer in press). Coalitions of males can reside in more than one pride (Bygott, Bertram & Hanby 1979) and distribute their time between neighboring prides (Schaller 1972). In contrast, nomads do not maintain a territory and move throughout the ecosystem (Schaller 1972). Lions from different social groups interact during territorial defense and at kills. Nomads can be seen as long distance disease dispersers, while shared males increase disease transmission between neighboring prides. A quantitative summary of lion population structure is summarized in Table 1 (Craft, *et al.* in prep).

The network model places $N_p = 180$ prides and $N_N = 180$ nomads at uniform random locations in a square region representing $A = 10,000 \text{ km}^2$ of the high lion density area of the Serengeti (Figure 1). The location of each pride is represented by a single point or *centroid* (geographical center of its territory). Prides are assigned to be adjacent to one another according to the estimated adjacency model (M_{adj}), and these adjacencies form the edges of the territory network (example in Figure 1B). A fraction of adjacent pairs (Ψ) are randomly assigned to have recently split from one another. Each pride is given a size (X_p) drawn from a best-fit gamma distribution. Contacts between prides occur at an average of $C_p = 4.55$ contacts per two-week period per pride, as estimated from a study in which 16 lionesses were observed continuously for a total of 2213 hours. Contacts between pairs of prides occur stochastically at rates that are weighted by a logistic function of their territory distance and whether they recently split (M_{contact}).

Coalitions of resident males and nomads are treated separately from prides of females and cubs. Male coalitions are represented as single units that increase connectivity between prides. Each territorial coalition belongs to either one or two prides; an estimated fraction η of all prides share their territorial coalition with one of their adjacent prides, and each remaining pride has a territorial coalition to itself. If a territorial coalition l is associated with $\eta_l > 1$ prides, it will be assigned to one of its prides and migrate from one (i) to another (j) with probability

$\mu_{ij} = \left(1 - \exp(-\zeta h)\right) / (\eta_l - 1)$ where h is a small time-step and ζ is the territorial male migration rate.

Nomadic lions are given group sizes (X_N) randomly generated from an estimated distribution and are assumed to migrate via a variance gamma process (M_{nomad}). Each group is initially assigned to the territory of a randomly selected pride, and at any point thereafter, resides in or around the territory of exactly one pride. In any

small time step h , a group of nomads will migrate from the territory of its current pride (i) to that of another pride (j) with probability given by

$$Z_{ij} = \left(1 - \left(1 - \left(F(d_{ij} + \alpha/2) - F(d_{ij} - \alpha/2) \right) \right)^h \right) / c_i$$

where $F(\)$ is the cumulative distribution function for displacement over a two-week period; d_{ij} is the distance between the centroids of territories i and j ; α is the average pride territory width ($\alpha = \sqrt{A/N_p}$); and c_i is a normalizer. Nomads are assumed to contact their local pride at a uniform rate derived from the average rate of pride-nomad contacts per pride (C_N).

When a pride contacts another pride or nomadic coalition, only a subset of the pride is actually involved in the interaction (G), and the number of lions involved is drawn randomly from an estimated distribution that depends on the size of that pride. Specifically, the log of group size increases approximately linearly with pride size (Table 1). When nomads contact prides, all members of the coalition are assumed to be present.

Epidemiological Model

We model disease dynamics using a stochastic SEIR (susceptible-exposed-infectious-recovered) approach. Lions frequently contact all other lions in their pride or nomadic group, so we assume that any given pride or group of nomads moves through the four disease classes as a unit, as in a Levins-type patch model (Levins 1969, Hanski & Gilpin 1997). A group is considered exposed when its first member becomes infected; the group transitions stochastically from exposed to infectious at a rate of 1/7 per day and from infectious to recovered at a rate of 1/14 per day.

When an infected group (A) contacts a susceptible group (B), the probability of disease transmission is a function of the number of individuals involved in the interaction and a per-contact transmissibility parameter (T), given by

$$\tau_{AB} = \sum_{j,k} p_j q_k \left(1 - (1 - T)^{j \cdot k} \right)$$

where p_j and q_k are the probabilities that the group sizes from A and B are j and k , respectively. This assumes that every lion in one group encounters every lion in the other group (recall that the expected size of a contact group is typically smaller than the size of the pride). When a susceptible coalition of territorial males resides with an infected pride, the coalition is immediately infected; and when an infected coalition of territorial males switches to a susceptible pride, it immediately infects the second pride.

Unless stated otherwise, the analysis is based on 200 simulated epidemics at 60 transmissibility values (T) between 0.0 and 0.3. For each run, a new lion population network was generated randomly, parameters were set to the values given in the *estimated quantities* column of Table 1, and the first pride infected was chosen at random from either the subset or the population as a whole. We conducted sensitivity analysis by running 100 replicate simulations at each of 10 transmissibility values using parameter values chosen randomly from the distributions given in the *distributions* column of Table 1 (S3).

Statistical Methods

Centrality analysis. For any given pride, distance to edge is calculated as the shortest Euclidean distance from its centroid to the ecosystem boundary; degree is simply the number of prides with adjacent territories; and closeness centrality is the reciprocal of the sum of shortest paths to all other prides in the population. We calculate shortest paths using the function `networkx.path.all_pairs_shortest_path_length` in the `networkx` software package <http://networkx.lanl.gov>. The centrality analysis presented in Figure 2 is based on 1400 epidemic simulations at $T = 0.10$, each based on a unique randomly generated lion population. For each centrality metric, we (1) calculated the average within the subset and the average overall for every simulation and conducted a Wilcoxon signed rank test on the data, and (2) binned prides into five equal-sized bins and calculated both the fraction of prides infected across all simulations and the fraction of all outbreaks originating at a pride within the bin that ultimately infected at least 50% of prides. Finally, we performed a full factorial logistic regression using the three centrality metrics as predictor variables (distance to edge, degree, and closeness

centrality) and the infection state of the pride (infected or not during an epidemic) as the response variable (Table S1).

Network correlograms. The network correlograms show the average correlation in infectious periods at each discrete network distance class. Each pride has a series of binary-valued disease states a_1, a_2, \dots, a_L , where $a_i \in \{0, 1\}$, where zero and one correspond to uninfected and infected days, respectively, and L is the length of the epidemic in days. For each simulation, let r_{xy} denote the Pearson product-moment correlation coefficient between these time series for every pair of prides x and y . The average correlation coefficient for network distance d in a given simulation is given by

$$\langle r_d \rangle = \frac{1}{|\Delta_d|} \sum_{(x,y) \in \Delta_d} r_{xy}$$

where Δ_d is the set of all pairs of prides that have minimum path length of d in the territory network and $|\Delta_d|$ is the number of pairs in that set. These calculations were adapted from the *R* library *ncf* (Bjornstad & Falck 2001).

The epidemiological data from the 1994 CDV outbreak are incomplete. For each of the 17 infected prides in the study area, the onset of infection was based on the date of first observed death or first confirmed seropositive individual in that pride, whichever occurred first. We discarded the date of onset for three prides that only provided serological evidence of infection, as infection had occurred at an indefinite time in the past. For each of the remaining prides, we stochastically reconstructed the disease state time series assuming that (1) pride infectious periods are random variables distributed exponentially with a mean of two weeks (as in our SEIR model) and (2) the date at which disease was first observed is selected uniformly from the infectious period of the pride. The network correlogram based on observed 1994 data (Figure 4A) gives averages over 1000 time series reconstructions. The network correlograms for the simulations were calculated using complete time-series data. The full population analysis includes all 180 prides, whereas the subset analysis is based on a randomly drawn sample of 15 prides (to replicate the incompleteness the empirical data).

Table 1. Demographic, contact, and epidemiological parameters for Serengeti lion prides.

Demographic parameters	Estimated quantities	Distributions**	Reference
A : area of ecosystem	10,000 km ²	N/A	(Packer 1990)
N_p : number of prides in ecosystem	180	$U(150,200)^*$	(Packer 1990)
X_p : pride sizes (number of females and cubs over three months old)	X_p : Gamma(k, θ) with $\theta = 4.707$, $k = 2.226$ Mean pride size = 10.48	θ : $N(4.707, 1.243)$ k : $N(2.226, 0.636)$	Pride Sheets (PS) 91-92
η : fraction of prides that share territorial males with one other pride	0.882	η : $N(0.882, 0.078)$	PS 92
ζ : rate at which territorial male coalitions switch prides	0.25 switches/day	ζ : $N(0.25, 0.070)$	B. Kissui, unpublished
M_{adj} : territory adjacency model	$\ln\left(\frac{P_{adj(AB)}}{1 - P_{adj(AB)}}\right) = 1.483 - 0.386 \cdot S_{AB}$ (S_{AB} = the number of prides located in the joint radius of A and $B^{\dagger\dagger}$) Mean number adjacent prides = 7.36	intercept $\sim N(1.483, 0.225)$ slope $\sim N(-0.386, 0.041)$	PS 91-92
Ψ : proportion of adjacent prides recently split from a common pride	0.063	$\Psi \sim N(0.063, 0.021)$	PS 85-87
N_N : # nomads	180	$U(150-200)^*$	PS 92
X_N : nomad group sizes	X_N : Log-normal(μ, σ) with $\mu = 0.292$, $\sigma = 0.446$ Mean group size = 1.51	$\mu \sim N(0.292, 0.065)$ $\sigma \sim N(0.446, 0.046)$	PS 92
M_{nomad} : nomad migration model Horizontal (x) and vertical (y) displacements per day are given by gamma distributions	Disp _x : Gamma(k_x, θ_x) with $k_x = 0.382$, $\theta_x = 2.85$ Disp _y : Gamma(k_y, θ_y) with $k_y = 0.714$, $\theta_y = 1.743$	$k_x \sim N(0.382, 0.029)$ $\theta_x \sim N(2.85, 0.02)$ $k_y \sim N(0.714, 0.029)$ $\theta_y \sim N(1.743, 0.019)$	M.C. unpublished
Contact parameters			

** Confidence intervals marked with an asterisk (*) are best guesses made by M.C. and C.P.

†† The joint radius of A and B is the union of two regions: (1) the semicircle with straight-edge centered at A that runs through B , and (2) the semicircle with straightedge centered at B that runs through A .

C_p : average rate of pride-pride contacts per pride	4.55 contacts/two weeks	$C_p : N(4.55, 0.573)$	PS 85-87
M_{contact} : contact weighting model	$\ln\left(\frac{w_{c(A,B)}}{1-w_{c(A,B)}}\right) = \alpha + \beta_d d_t(A, B) + \begin{cases} -\beta_s & \text{if recently split} \\ \beta_s & \text{otherwise} \end{cases}$ $w_{c(A,B)}$ is the weighting factor for the contact rate between A and B ^{**} $d_t(A, B)$ is the territory distance between the prides.		PS 85-87
α : intercept	3.265	$\alpha : N(3.265, 0.371)$	PS 85-87
β_d : network distance coefficient	1.698	$\beta_d : N(1.698, 0.264)$	PS 85-87
β_s : recent split coefficients	0.696	$\beta_d : N(0.696, 0.220)$	PS 85-87
C_N : average rate of pride-nomad coalition contacts per pride	7.136 contacts/two weeks	$N(7.136, 1.018)$	PS 85-87
G : pride group size during contact $G' = \log(G + 1)$	$G' \sim N(\mu_{G'}, \sigma_{G'})$ with $\mu_{G'} = 0.447 + 0.014 \cdot X$ $\sigma_{G'} = 0.232$ Mean group size = 3.65	$\mu_{G'}$ intercept $\sim N(0.447,$ $\mu_{G'}$ slope $\sim N(0.014, 0,$ $\sigma \sim N(0.232, 0.022)$	PS 85-87
Epidemiological Parameters			
ε : incubation period (days)	ε : Exponential(λ) with $\lambda = 1 / 7$	N/A	(Appel 1987)
ι : infectious period (days)	ι : Exponential(λ) with $\lambda = 1 / 14$	N/A	(Greene & Appel 2006)

** Specifically, $w_{c(A,B)}$ is the estimated probability that pride A will contact pride B per daylight hour of observation of A.

FIGURES

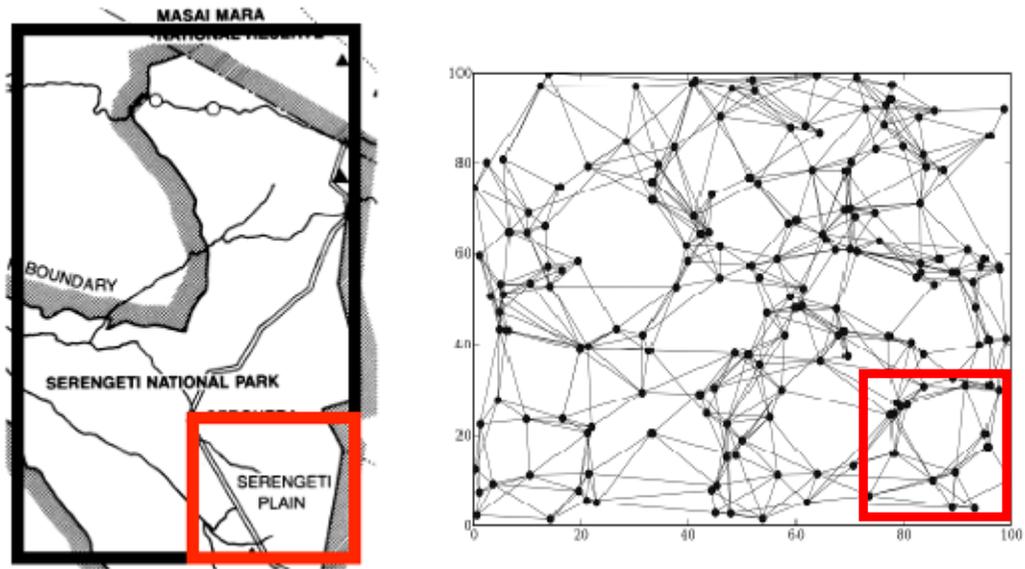


Figure 1. The ecosystem and study area (subset) in both the Serengeti and the model. (A) The Serengeti ecosystem (black rectangle: suitable lion habitat; red square: SLP study area). (B) A simulated lion population based on estimates of territory locations and adjacencies from SLP data (black rectangle: model ecosystem; red square: sampled subset). Nodes represent prides and edges indicate prides with adjacent territories.

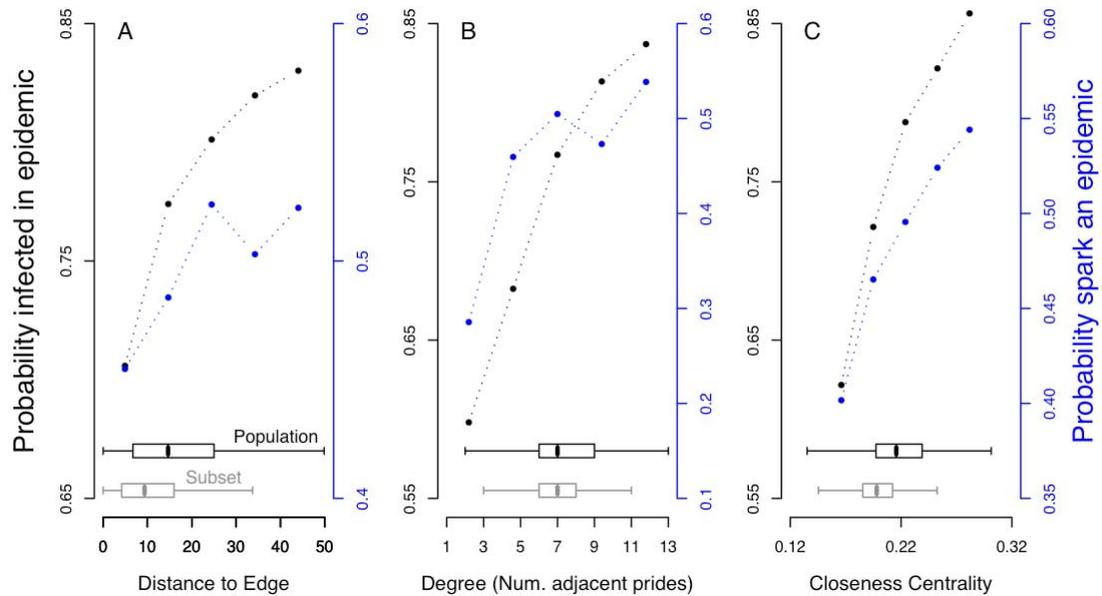


Figure 2. Epidemiological risk versus the geographic and network location of a pride. At $T = 0.10$, distance to (A) edge, (B) degree, and (C) closeness centrality all positively correlate with each other, with the probability that a pride will become infected during an epidemic (black dotted lines), and with the probability that the pride will spark an epidemic if it is the first to be infected (blue dotted lines). An epidemic is defined as any outbreak that reaches at least 50% of prides. Each graph is based on 1400 simulations. Box plots show the distributions of these values for the entire population (black) and the subset (gray), excluding outliers beyond the median $\pm 1.5 \cdot \text{IQR}$.

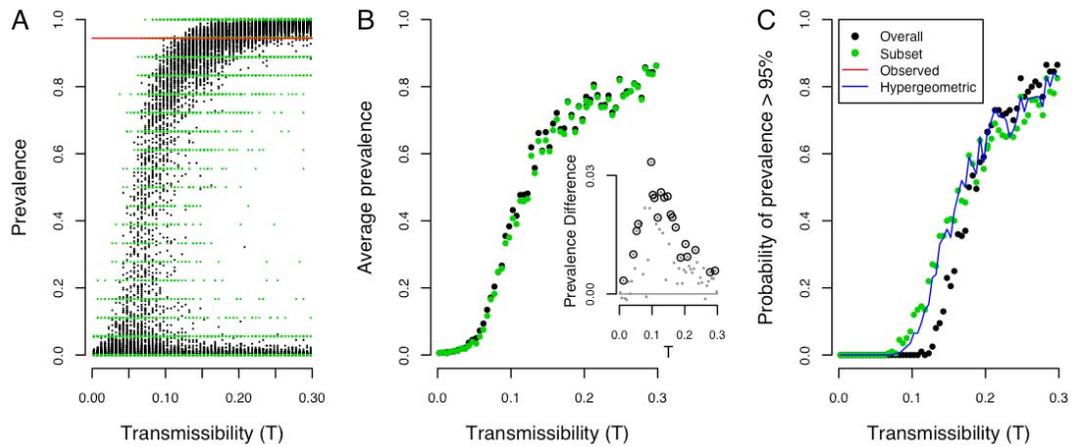


Figure 3. The prevalence of CDV in the population and subset as a function of transmissibility. (A) Prevalence over a range of transmissibility values in the entire population of 180 prides (black) and the subset of 18 prides (green). Each point represents the results of a single simulation. The red line is the prevalence observed in the 1994 CDV outbreak, as estimated from 18 prides in the SLP study area. (B) Average prevalence in the population (black) and subset (green) over a range of transmissibility values. Inset: difference between overall prevalence and subset prevalence. Circled dots are statistically significant (paired t-test, $P < 0.05$). (C) Probability of a large outbreak (>94% prides infected) over a range of transmissibility values for the population (black) and subset (green), compared to null expectations for the subset based on a hypergeometric model (blue line). The null values were generated by drawing a single hypergeometrically distributed random number for each simulation, with parameters $N = 180$, $n = 18$, $m =$ total number of prides infected in the simulation. Probabilities were averaged across all simulations at each transmissibility value.

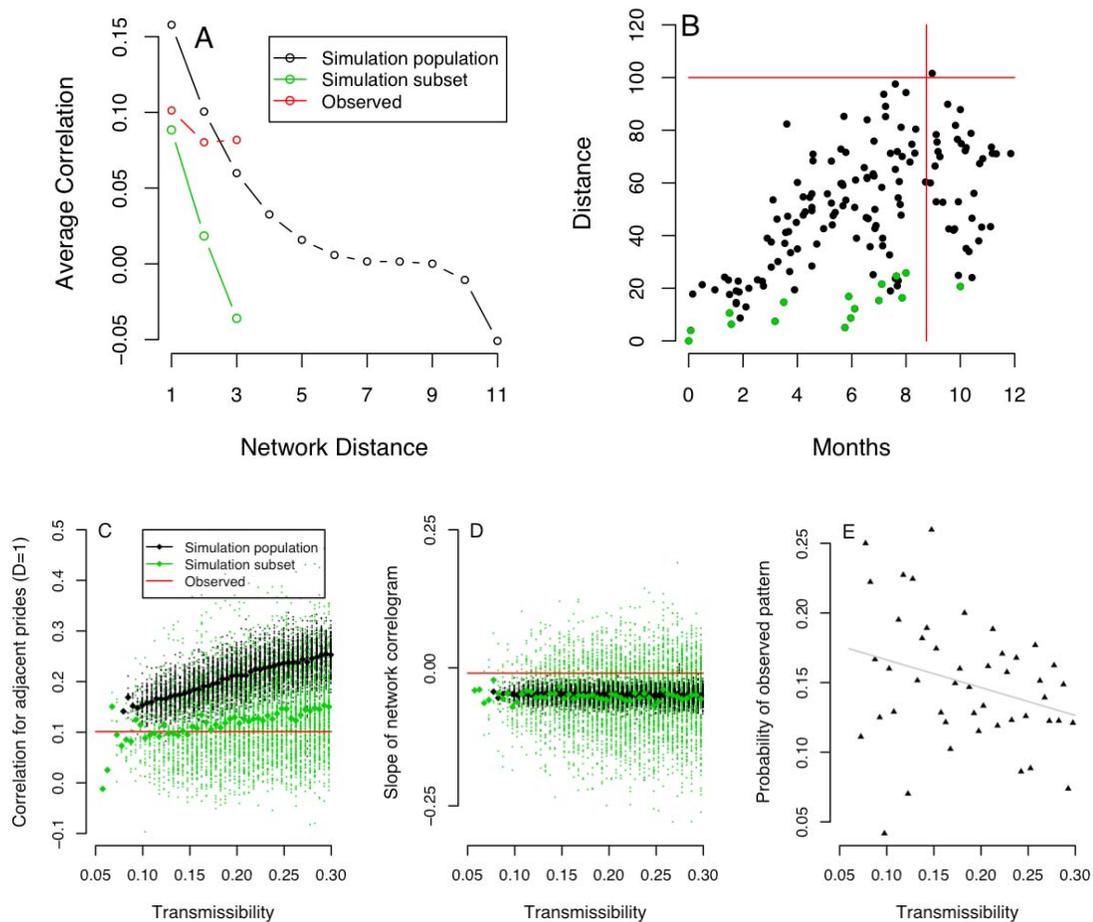


Figure 4. Spatial spread of CDV. (A) Network correlograms for simulated and observed epidemics. In simulated epidemics (with $T = 0.1725$), the average correlation in the timing of infectious periods between randomly chosen prides decreases with increasing network distance. Correlations between adjacent prides were lower in both the observed 1994 CDV outbreak (red) and simulated subsets (green). (B) Representative example of a simulated epidemic that began in the subset and swept through the entire population, occasionally returning to the subset. Points indicate the time and distance from first infection of each infected pride (green: subset prides, black: other prides). Red lines represent the observation that the 1994 CDV epidemic took 35 weeks to reach 100 km from the study area. (C) Average correlation in infectious period for all directly adjacent prides in the subset (green) and population (black). Small points

show averages from individual simulations and large points show overall means at each transmissibility. The red line is the estimated correlation from the 1994 outbreak. (D) Slope of the network correlograms for the subset (green) and population (black). Small points show slopes from individual simulations and large points show mean slope across all simulations. Red line is the estimated slope from the 1994 outbreak. (E) The probability that the observed (1994) correlogram would arise from the model across transmissibilities. This probability is the fraction of simulations that lay both below the red line in panel C and above the red line in panel D. Line is the least-square linear regression line ($P < 0.05$).

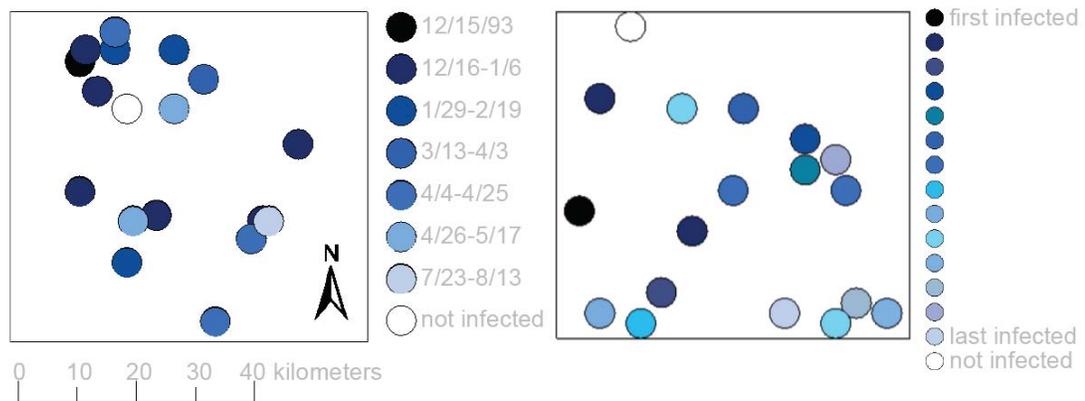


Figure 5. Spatio-temporal progression of CDV in both the observed study area and a model subset. Disease moves through prides in (A) the observed study area during the 1994 outbreak (the timing of a pride’s infection corresponds to the first date that an infected or seropositive lion from the pride was observed) and (B) a simulated epidemic with $T = 12.75$. The units of time are weeks. The black circle shows the first pride infected and color changes from dark blue to light blue as the epidemic progresses. Empty circles indicate uninfected prides. The rest of the ecosystem would extend to the left and top of each pictured subset as in Figure 1A and 1B.

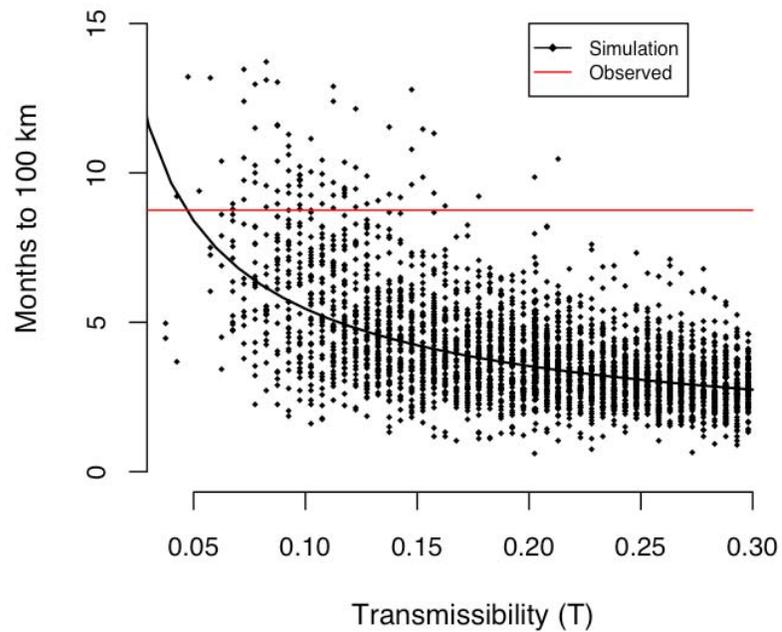


Figure 6. Epidemic velocity. Each point represents the time until the disease reached 100 km from the first infected pride for a single simulated epidemic starting at a randomly chosen pride in the subset. The black line shows the least squares linear regression on log-log transformed values. The red line shows the estimated velocity for the observed 1994 outbreak.

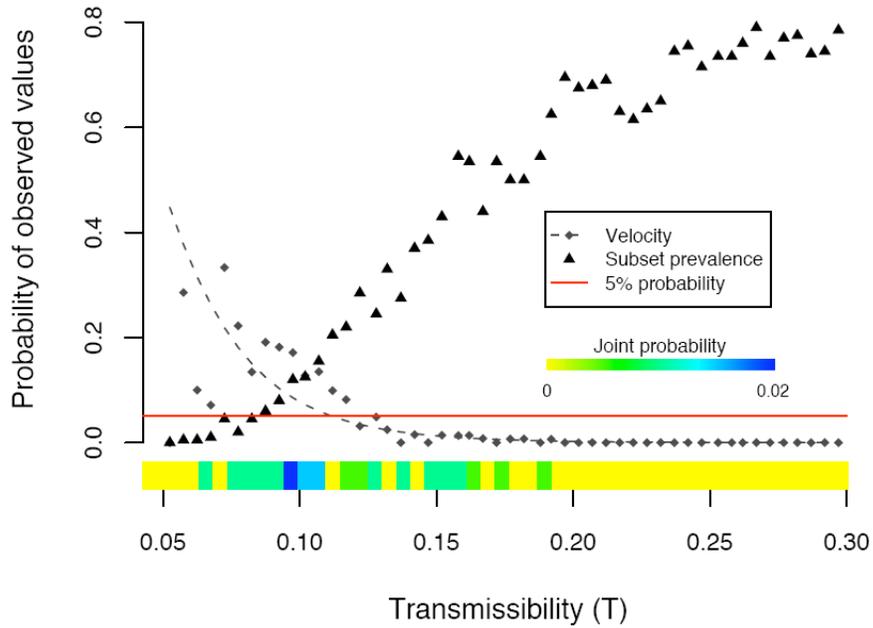


Figure 7. Probability of observed epidemiological patterns in a simulated outbreak maintained solely by lion-to-lion transmission. The probability of the observed velocity is calculated as the fraction of simulations that took at least 35 weeks to reach 100 km. The probability of the observed prevalence is calculated as the fraction of simulations that infected at least 17 of the 18 prides in the subset. The red line at probability 0.05 indicates that there is a very limited range of transmissibility at which both patterns have at least a 5% of occurring. The joint probability is calculated as the fraction of simulations that exhibited both the observed velocity and prevalence.

Supporting information

S1. Geographic and network location versus the probability that a pride is infected during an epidemic. Table S1 gives the results of the multivariate logistic regression analysis for distance to edge, degree, and closeness centrality vs. the probability that a pride is infected during an epidemic. Grey rows highlight significant factors.

Source	df	Likelihood-ratio chi-square	<i>P-value</i>
Distance to edge (<i>DE</i>)	1	0.09326	0.7601
Degree (<i>Deg</i>)	1	118.5862	<.0001
Closeness Centrality (<i>CC</i>)	1	28.5187	<.0001

Table S1. Logistic regression of population structure on epidemiological risk.

S2. Sensitivity analysis of model based on 100 replicate simulations at each of 10 transmissibility values. For each simulation, we randomly drew all parameter values from the ranges given in Table 1. Figure S1 was calculated from the results of these simulations, using the same methods as described for Figure 7. The qualitative and quantitative agreement between the two figures show that the basic conclusion of the paper – that lions probably did not sustain the 1994 CDV epidemic themselves – is robust to uncertainties in the parameters.

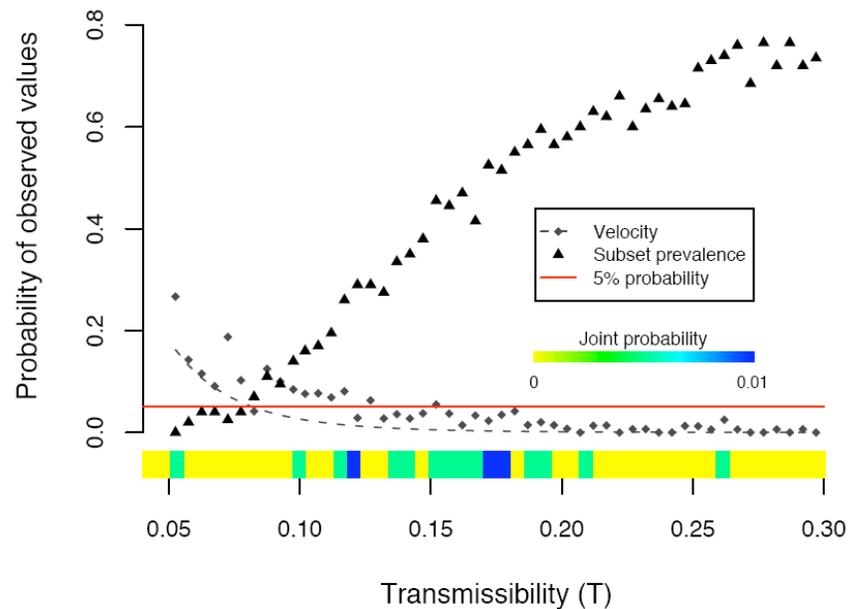


Figure S1. Probability of observed epidemic values across a range of transmissibilities. The probability of the observed velocity is calculated as the fraction of simulations that took at least 35 weeks to reach 100 km. The probability of the observed prevalence is calculated as the fraction of simulations that infected at least 17 of the 18 prides in the subset. The red line at probability 0.05 indicates that there is a very limited range of transmissibility at which both patterns have at least a 5% of occurring. The joint probability is calculated as the fraction of simulations that exhibited both the observed velocity and prevalence. These calculations are based on simulations in which parameter values are randomly drawn from the estimated distributions in Table 1.

CHAPTER 4

Dynamics of a multihost pathogen in a carnivore community^{§§}

Summary

1. We provide the first theoretical analysis of multihost disease dynamics to incorporate social behavior and contrasting rates of within- and between-group disease transmission.
2. A stochastic susceptible-infected-recovered (SIR) model of disease transmission involving one to three sympatric species was built to mimic the 1994 Serengeti canine distemper virus outbreak, which infected a variety of carnivores with widely ranging social structures. The model successfully mimicked the erratic and discontinuous spatial pattern of lion deaths observed in the Serengeti lions under a reasonable range of parameter values, but only when one to two other species repeatedly transmitted the virus to the lion population.
3. The outputs from our model suggest several principles that will apply to most directly transmitted multihost pathogens: (i) differences in social structure can significantly influence the size, velocity, and spatial pattern of a multihost epidemic; and (ii) social structures that permit higher intraspecific neighbor-to-neighbor transmission are the most likely to transmit disease to other species; whereas (iii) species with low neighbor-to-neighbor intraspecific transmission suffer the greatest costs from interspecific transmission.

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Introduction

Multihost pathogens are likely to exhibit different spatiotemporal dynamics than pathogens that only infect a single host species. From one perspective, multiple hosts could be considered an additional form of heterogeneity that divides the total host population into subpopulations, between which transmission occurs at a different rate than within each subpopulation. Single-species “subpopulation” approaches (with multiple scales of mixing) have been successfully developed to examine disease transmission between sexes in the case of sexually transmitted diseases (May & Anderson 1987, Anderson 1991); between children of different ages (measles, mumps, rubella) (Anderson & May 1985); people living in regions, cities, and villages of different sizes (measles, influenza) (May & Anderson 1984, Grenfell & Bolker 1998, Grenfell, Bjornstad & Kappey 2001, Viboud *et al.* 2006); and hosts living as a metapopulation in different patches of habitat (Swinton *et al.* 1998, McCallum & Dobson 2002, McCallum & Dobson 2006).

However, using subpopulation approaches on multihost pathogens is not as straightforward as it seems; different host species might vary in their response to infection, have varying contact patterns based on social behavior, and have different spatial distributions across the landscape (Dobson 2004). Due to these complexities, previous work on multihost models has made simplifying assumptions and assumed that each host population is well mixed, and specifically ignored heterogeneities due to social organization (Dobson 2004, Fenton & Pedersen 2005, McCallum & Dobson 2006). We have, therefore, developed a general stochastic, spatial model of a disease outbreak in two and three host-species communities with widely ranging social structure. Our model structure is based on a 1994 outbreak of canine distemper virus (CDV) in the Serengeti ecosystem that killed one-third of the lion population (*Panthera leo*) (Roelke-Parker *et al.* 1996, Kock *et al.* 1998, Packer *et al.* 1999). CDV is a contagious multihost virus spread by aerosol inhalation, which affects all carnivore families. Infected animals either die or obtain lifelong immunity (Appel 1987, Williams 2001).

Because lions are territorial, and most opportunities for disease transmission between social groups involve immediate neighbors (M.E.C., unpublished data), the erratic and discontinuous spatial pattern of CDV spread in the 1994 epidemic seems unlikely to have resulted solely from lion-to-lion transmission (Fig. 1). During the 1994 outbreak, the same CDV variant was responsible for deaths in spotted hyenas (*Crocuta crocuta*) (Haas *et al.* 1996, Roelke-Parker *et al.* 1996, Carpenter *et al.* 1998), while jackals (*Canis adustus*, *Canis aureus*, *Canis mesomelas*) also showed CDV-like symptoms and subsequently tested positive for CDV antibodies (Alexander *et al.* 1994, Roelke-Parker *et al.* 1996).

Hyenas and jackals had the potential to transmit CDV to lions, as the two species are more abundant than lions (Campbell & Borner 1986), and frequently interact with lions at kills (Schaller 1972, Cleaveland *et al.* 2008). While lions, hyenas, jackals, bat-eared foxes (*Otocyon megalotis*) and potentially many other carnivore species (e.g. leopards, *Panthera pardus*) were affected by the 1994 CDV outbreak (Roelke-Parker *et al.* 1996), our most detailed data come from the long-term monitoring of the Serengeti lions (Packer *et al.* 2005). We therefore treat lions as the sentinel species when comparing the observed pattern of infection in the 1994 lion population with the model's CDV spatial spread.

Questions

We developed a stochastic simulation model to capture the general spatial and temporal patterns observed in the 1994 CDV outbreak. Although the model is based on the lion outbreak, it has been developed to provide more general insights into disease outbreaks in other communities, where multiple host species are susceptible to infection by the same pathogen. In particular, we ask whether differences in territorial social structure affect the spatial and temporal pattern of disease outbreaks, and if the time course of the epidemic is sensitive to different rates of within- vs. between-species interaction. Social organization due to territorial behavior divides intraspecific transmission into two major components: within and between groups. Within-group transmission can occur during normal social interactions (feeding, grooming), whereas between-group transmission

can occur during fights over food and territory, or during immigration events. Interspecific transmission occurs when multiple species feed together or during intraguild predation events.

We performed a set of simulations that examine the epidemic dynamics of a directly transmitted pathogen involving multiple host species with contrasting social organizations (e.g. isolated vs. well connected territorial structures), characterized by different within- and between-group transmission rates. After exploring the epidemic dynamics for each species in isolation, we examine the consequences of coexistence between pairs of species using high and low rates of interspecific transmission. Finally we ask whether the coexistence of three hosts differs in any substantive way from any two-species scenario.

We use the simulation to ask:

- How do within- and between-group contact patterns affect the incidence, rate of spread, probability, and spatial pattern of infection in multiple hosts with coexisting pathogens?
- How do the model results compare with the observed outbreak?

Modeling Approach

The model describes the spatial and temporal dynamics of a pathogen in a spatially structured, multihost community. The habitat is divided into a two-dimensional grid of 625 patches, with each patch containing a local population of each species. Because of the natural boundaries of the Serengeti ecosystem, we chose not to wrap the edges of the simulated habitat. Infection is spread within local populations, between different species occupying the same patch, and between any populations/species occupying the eight neighboring patches. The pathogen is modeled in a stochastic, density-dependent, susceptible-infected-recovered (SIR) framework. The model was programmed in C.

The importance of group size to pathogen persistence is well known (Swinton *et al.* 2001, Park, Gubbins & Gilligan 2002, McCallum & Dobson 2006), so we held group size constant across species and across social groups in order to isolate the effect of social organization. Each patch begins with 10 individuals of each species. An

individual may be categorized in one of 3 states: S (susceptible), I (infected) or R (recovered). All individuals, except an initially infected source, begin the simulation in state S . Transitions occur from $S \rightarrow I$ (infection) and from $I \rightarrow R$ (recovery). During each time-step, we determine the probability of a susceptible individual becoming infected, $p_{S \rightarrow I}$, and of an infected individual recovering (either dying or obtaining lifelong immunity), $p_{I \rightarrow R}$. The number of actual transitions is drawn from a binomial distribution, $B(n, p)$. For the infection transition, n is the number of susceptible individuals in the group, while for the recovery transition, n is the number of infected individuals.

The probability that a susceptible individual, i , will be infected depends on the number of infections in its own social group, interspecific transmission within the same patch, and intra- and interspecific transmission from neighboring patches. Two ‘who acquires infection from whom’ matrices (WAIFW; Anderson & May 1991) characterize the force of infection between individuals of each group; let $\beta_{W,ij}$ represent within-patch transmissions and $\beta_{B,ij}$ represent between-patch transmissions). The total probability of infection is given by:

$$1 - \exp \left[- \left(\sum_{j \in S_L} \beta_{W,ij} I_j + \sum_{j \in S_N} \beta_{B,ij} I_j \right) \right],$$

where S_L is the set of groups sharing the local patch and S_N represents the groups in neighboring patches and I_j is the number of infected individuals in group j . Each infected individual has a fixed probability, μ , of recovering.

Interspecific β values are taken as a weighted average of the intraspecific values so that

$$\beta_{ij} = \beta_{ji} = \frac{1}{2} c (\beta_{ii} + \beta_{jj}),$$

where c describes the level of interspecific interactions (or coupling). We used two different values of c , designated “high” and “low” (0.2, 0.01, respectively) for the multi-species simulations.

The value of the average reproductive rate of the pathogen is defined as R_0 . In general a pathogen can only persist when R_0 is >1 (when each infected individual

infects at least one other individual). Species' within- and between-patch transmission rates were chosen so that the R_0 values in a single-species habitat equaled 2.2. CDV is closely related to phocine distemper virus, for which the empirically estimated R_0 is 2.8 (Swinton *et al.* 1998). Different social systems were modeled by choosing different relative rates of within- and between-group transmission (Table 1).

In the Serengeti, the African lion lives in territorial social groups (prides) consisting of related females and their dependent offspring. Before the 1994 epidemic, average pride sizes (excluding cubs <3 months) were 10 individuals (M.E.C. unpublished data) defending territories ranging from 15 to 150 km² (Mosser 2008). Lions form fission-fusion groups where pridemates are in frequent physical contact, but only occasionally contact their neighbors during territorial defense or fights over food (Schaller 1972, M.E.C, unpublished data). Thus the within-patch (or within-pride) transmission rate for lions will be far higher ($R_0 > 1$) than between-patch transmission ($R_0 < 1$).

The spotted hyena lives in social groups (clans) averaging about 45 individuals per clan (Hofer & East 1995). These hierarchical clans consist of related females and immigrant males who defend exclusive group territories (16-55 km²) and encounter their neighbors during territorial clashes, or when feeding at the same carcass (Hofer & East 1993a). Additionally, Serengeti hyenas have a unique feeding adaptation where they commute to migratory prey and associate with non-clan members at waterholes and resting sites (Hofer & East 1993b). Thus hyenas are expected to have high within-patch transmission (but contact each other less than lions), as well as high between-patch transmission.

Jackals live in small family groups of two to four who are in close contact with each other (Moehlman 1983). Serengeti golden and black-backed jackals actively defend discrete territories ($\approx 2-4$ km²) from neighbors; they also make extraterritorial forays to water sources and large mammalian kills (Moehlman 1983). We therefore consider each "patch" of 10 individuals to consist of two to five loosely connected groups of jackals. Although they interact with each other less frequently than

pridemates, jackals contact individuals from neighboring patches more frequently than do lions.

Infections were introduced in a single individual at the edge of the grid to mimic a pathogen introduced from domestic dogs at the edge of the park (Cleaveland *et al.* 2000). We ran 150 simulations for each combination of species. To check whether changes in disease dynamics were due to social structure, rather than to a simple increase in overall population size, we ran controls where the same species was coupled with itself within separate partitions of the same patch. Each simulation ran until all infections disappeared. For each species, we also varied the within- and between-group transmission rates to confirm that the results presented here were representative of the overall range of possible outcomes.

We used the package NCF (Bjornstad & Falck 2001) for R (R Development Core Team, 2006) to evaluate the spatial pattern in both the simulated and observed outbreaks. For each time-step (day) in the simulated outbreaks, we entered the number of active infections per grid square (pride) into the nonparametric correlation function (ncf). Because of the coarse-grained resolution of within-pride mortality in 1994, we constructed within-pride epidemic curves from the simulated outbreaks by aligning the simulated start dates, averaging the number of infections at each time-step, and rounding the values into discrete integers. We combined these simulated within-pride epidemic curves with the observed first death date per pride and spatial location, to create a complete time-series for the observed outbreak.

Results

Single-species models.

Depending on contact structure, single-species epidemics produced epidemic curves that varied in impact (average cumulative number of infected hosts by the end of an outbreak), velocity (cumulative number infected per unit time), and probability and persistence of an outbreak (Figs 2 and 3). The outbreaks in hyenas produced the most infected individuals, spread with the highest velocity, and had the highest percent of runs with epidemics (defined as lasting longer than 200 time steps). In contrast, lions

had the fewest infected individuals and slowest velocity; the disease generally burned out (few runs caused epidemics, and those that did were of shorter duration). Jackals produced values intermediate between lions and hyenas, except that infection persisted the longest in jackals (Fig. 2).

Multi-species models

Compared with single-species models, any representation of a multihost system inevitably involves an increased number of susceptible hosts with a concomitant effect on disease transmission and persistence. We isolated the impact of an increased number of susceptibles by constructing a series of controls that effectively doubled or tripled the number of individuals in the single-species simulations. We could then highlight the effects of social system *per se* by contrasting a lion-plus-lion model (which doubled the number of lions) to a lion-plus-hyena model (with the same number of individuals as the doubled-lion model, but with two different social systems).

Do within- and between-group contact patterns influence the impact of a pathogen?

Adding a second or third host species (Figs 2 and 3a) increased the impact of the pathogen (average cumulative number of infected individuals in the first host species), although this was not always significant (see Supplementary material). For example, the number of infected hyenas did not increase significantly when hyenas were weakly coupled with another species, even to an overlapping control population of hyenas. However, many more lions were infected when weakly coupled with either hyenas or jackals than with a control population of lions. Note, though, that fewer jackals are infected when lions are weakly coupled with jackals, compared to the weakly coupled doubled-jackal control. This is due to the dilution effect of “wasting” infections on less competent transmitters such as lions (Ostfeld & Keesing 2000). An amplification effect can be seen when hyenas (the most competent transmitters) are paired with lions, compared to the lion-plus-lion scenario. With high interspecific connectivity, the overall increase in infecteds can largely be attributed to increased population size, because the

doubled and tripled single-host-species scenarios are indistinguishable from the two- and three-host-species outputs.

Do within- and between-group contact patterns influence the rate of spread of the pathogen through the system or the probability of an epidemic?

When additional species were added to a single-species epidemic with high coupling, the average velocity (number of infecteds per unit time) of the wave front increased, and there was a higher probability of an epidemic; but this was not always the case when species were loosely connected (Fig 3b,c). For example, in hyenas, the velocity of infection and probability of an epidemic actually slowed down when weakly combined with one or two additional species. The controls illustrate that at high coupling, there are large effects of adding any additional species (regardless of their social structure); but at low coupling, the social structure of the additional hosts can increase or decrease the velocity or probability of a large-scale epidemic.

Do within- and between-group contact patterns change the spatial spread of a pathogen?

Spatial spread of single-species infections differed according to contact patterns (Fig. 4a). While the epidemic always travels in a wave-like pattern, the neighbor-to-neighbor transmission rate determined the extent of spatial spread.

Hyenas and jackals have high conspecific neighbor transmission, so there is extensive spatial spread no matter which other species is added to their community. Low neighbor-to-neighbor transmission in lions, however, limits the spatial spread of the pathogen unless the lions are tightly coupled with another species. When lions are loosely coupled with another species, occasional spill-overs from the more competent host cause smaller local outbreaks (Fig. 4b).

Overall, the finer resolution of spatial spread in two-host systems depended on the level of connectivity between species. With low coupling, most cells were infected by conspecific neighbors causing long chains of same-species infection; fewer cells were infected. With high coupling, each species had a relatively equal chance of being

infected by a different species, and more cells were infected (Fig. 4b). When the spatial nonparametric correlation function was plotted at low and high coupling, the spatial correlation was consistently higher with high coupling (Fig. 4c), indicating a more coherent, wave-like spread of infection. With the low coupling, correlation between infection times broke down only a few cells away, confirming a more local, patchy spread.

When all three species were loosely coupled together, the wave-like pattern was replaced by disconnected jumps in the spatial pattern of infection and uneven coverage of infection when viewed from the lion's perspective (there was still a strong wave formation in jackals and hyenas) (Fig. 4b). As in the two-host case, most cells were infected by their conspecific neighbor. But with high mixing, there was a high coverage of infecteds, most infections stemmed from interspecific contacts, and spatial pattern was more of a multi-species wave of infection than in the two-species case, although the timing of infection in lions was still slightly patchy. The ncf also showed higher correlation with high coupling, and less correlation with low coupling.

In addition, when we used different within- and between-group mixing parameters (Species 1: 1.1, 1.1; Species 2: 0.5, 1.7; Species 3: 1.7, 0.5), our findings were consistent with the results obtained from the mixing parameters used in this model. Specifically, with the varied set of mixing parameters, we also found that differences in social structure can significantly influence the size, velocity, and probability of a multihost epidemic, especially with low interspecific coupling.

Comparison with observed outbreak

The low-coupling simulations generated spatial patterns that were more similar to the non-wavelike, patchy spread of CDV observed in the Serengeti lions. High-coupling models, on the other hand, generated an obvious wavelike pattern with a high degree of spatial correlation that contrasted sharply with the observed outbreak (Fig. 4c).

Discussion

These results have implications that extend beyond pathogens of Serengeti carnivores. Our model suggests a number of general principles that will apply to most directly transmitted pathogens, which can infect multiple host species: (1) differences in social structure can significantly influence the size, velocity, and probability of a multihost epidemic; (2) social structures that permit higher intraspecific neighbor-to-neighbor transmission are the most likely to transmit disease to other species; and (3) species with low neighbor-to-neighbor intraspecific transmission are most vulnerable to interspecific transmission.

Deterministic models by Holt & Pickering (1985); Begon and Bowers (1994); Dobson (Dobson 2004); Woolhouse, Taylor & Haydon (2001); and Dobson (2004) have consistently emphasized the importance of multiple scales of mixing, specifically the relative rate of within- vs. between-species transmission in determining the transient dynamics of infection. When interspecific transmission is high, our stochastic spatial model shows that the presence of multiple-host species is essentially equivalent to a larger susceptible host population. More hosts are infected, and the pathogen may have a significantly higher impact in species that could not sustain an outbreak in isolation. The combined population of species essentially acts as a single super species, incorporating the strongest parameters of each species. Thus the rate of disease spread can increase with the number of co-existing host species; the rate of interspecific transmission increases the cumulative number of hosts infected in all susceptible host populations; the probability of an extensive outbreak increases; and the number of individuals infected (and potentially dying) may be higher in host populations that would otherwise be too small or too dispersed to sustain the pathogen by themselves. Furthermore, adding a second species that is more effective at transmission produces an amplification effect; while a less-effective second species can cause a dilution effect (Keesing, Holt & Ostfeld 2006).

In the observed 1994 outbreak, hyenas and/or jackals could have feasibly acted as amplifying species by spreading the CDV through the more isolated lion prides and causing long-distance leaps in infection among prides. When we compared the observed

CDV outbreak to the simulations, results were reasonably similar to the low transmission-rate scenario. Based on our simplified model, we cannot say whether an outbreak restricted to hyenas, jackals and lions, or a larger combination of susceptible species (e.g. leopards, bat-eared foxes), could have created the observed outbreak, but rather that low interspecific contact rates feasibly could have accounted for the extensive coverage of CDV infection and erratic spatial spread seen in the Serengeti lions.

Multihost pathogens have particular importance for the management of endangered species. First, numerically abundant species will usually act as reservoirs of infection for endangered species that are, by definition, rare (McCallum & Dobson 1995, Funk *et al.* 2001, Woolhouse, Taylor & Haydon 2001). Second, infections would normally die out in any single-species system where the host experiences low levels of intergroup contact, but the risk of a persistent outbreak increases dramatically when it is exposed to a well mixed host species. Disease threats from sympatric species have historically been overlooked when considering reintroduction and translocation of social carnivores (focusing instead on the negative effects of kleptoparasitism and intraguild predation) (Gusset *et al.* 2008). But any highly territorial species will be especially susceptible to multihost diseases in the presence of less sedentary species such as hyenas or evenly distributed species such as jackals. These risks should be considered when translocating territorial social species for reintroductions.

The following supplementary material is available for this article online (Table S1).

Tables

Table 1. Relative rates of within- and between-group transmission

Resembles	R ₀ within-group	R ₀ between-group
Lion	>1 (1.9)	<1 (0.3)
Hyena	>1 (1.1)	>1 (1.1)
Jackal	>1 (1.5)	<1 (0.7)

The within- and between-R₀ values are calculated by: $n(1 - e^{-\frac{\beta}{\mu}})$, where n is the number of susceptible individuals that might be contacted by the initially infected individual, β is the infection rate per susceptible individual, and μ is the recovery rate. The model treats transmission from the initial infected to each susceptible as an independent Poisson process with rate β and duration $1/\mu$. The probability that each susceptible individual is infected is then $p_i = 1 - P[\text{no infection}]$, and the expected total number is np_i . $n_{\text{local}} = 9$; $n_{\text{nhbr}} = 80$; $\mu = 0.1$.

Figures & Legends

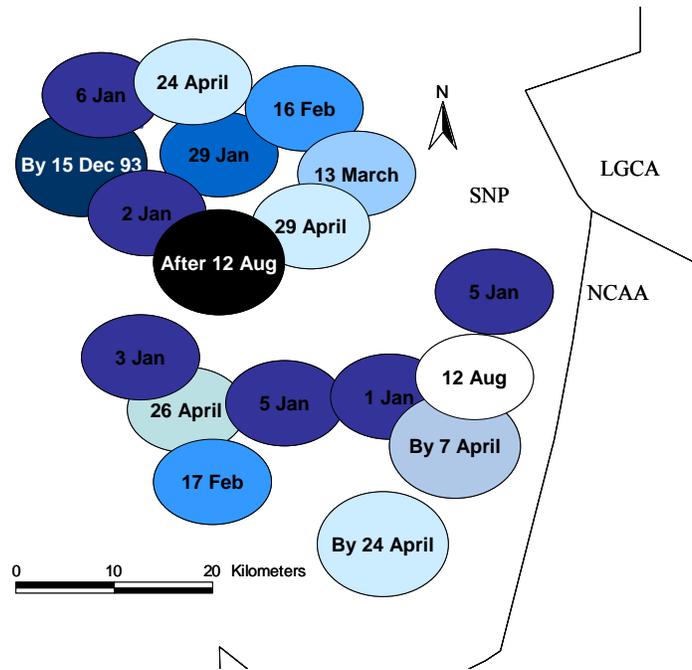


Figure 1. The observed dynamics of a canine distemper outbreak in the Serengeti lion study population in the southeast Serengeti National Park (SNP) near the Ngorongoro Conservation Area Authority (NCAA) and Loliondo Game Controlled Area (LGCA). Each oval represents a lion pride; the time course was determined either by a) the date of first observed death in a pride or b) by the date of sampling for the first seropositive individual in the pride. Prides infected early in the epidemic are colored dark blue, those infected later in the epidemic grade through to white. One pride remained uninfected (black).

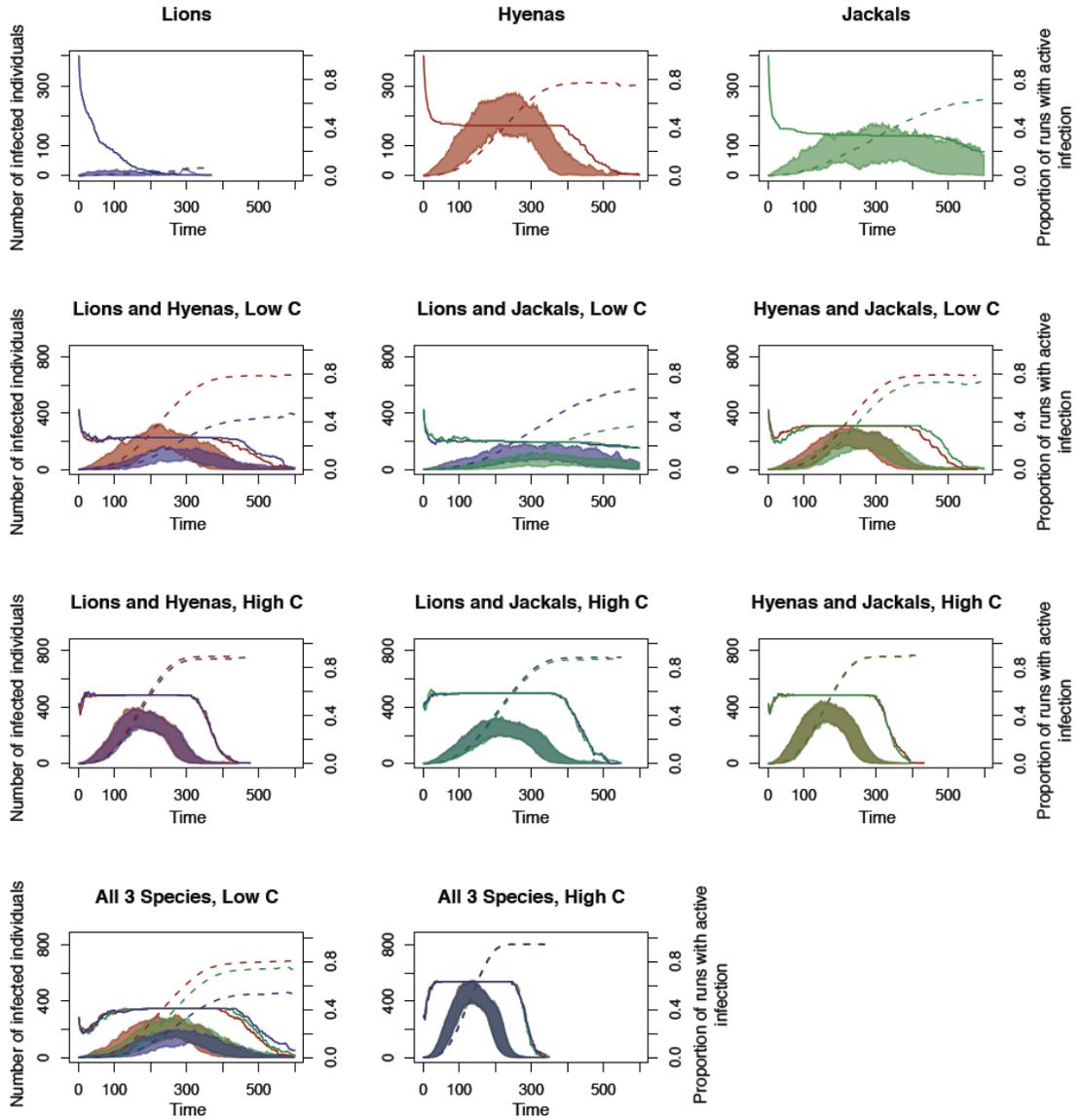


Figure 2. Temporal dynamics of simulated epidemics. Single species epidemics in lions, jackals, and hyenas and multiple species epidemics when co-existing species are weakly vs. highly coupled (low C vs. high C). Colored zones indicate the 10-90% quantiles of the number of infecteds in each species in runs where infections were still present (left y-axis). Solid lines, proportions of runs with an infection still present (right y-axis). Dashed lines, cumulative proportion of individuals that became infected during the course of the epidemic. Population size for each species, 6250 individuals.

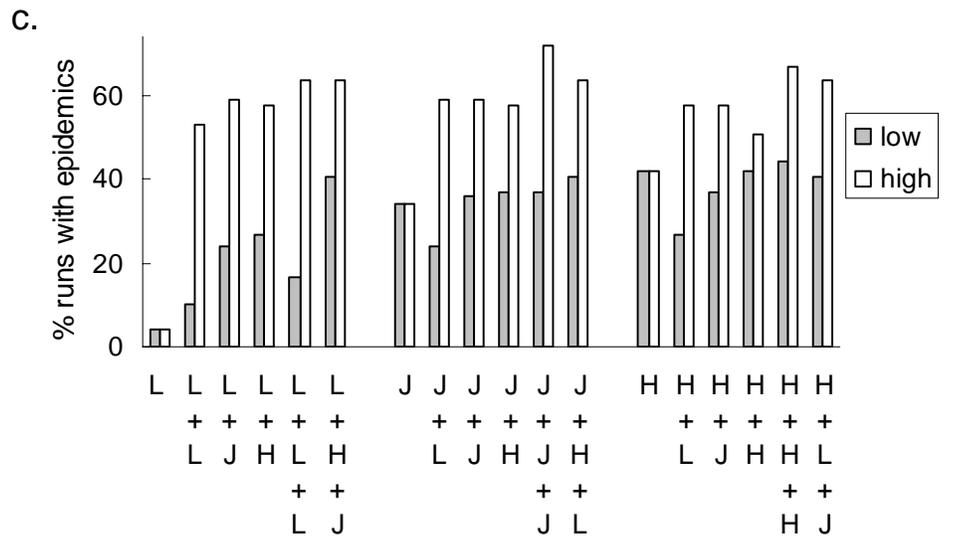
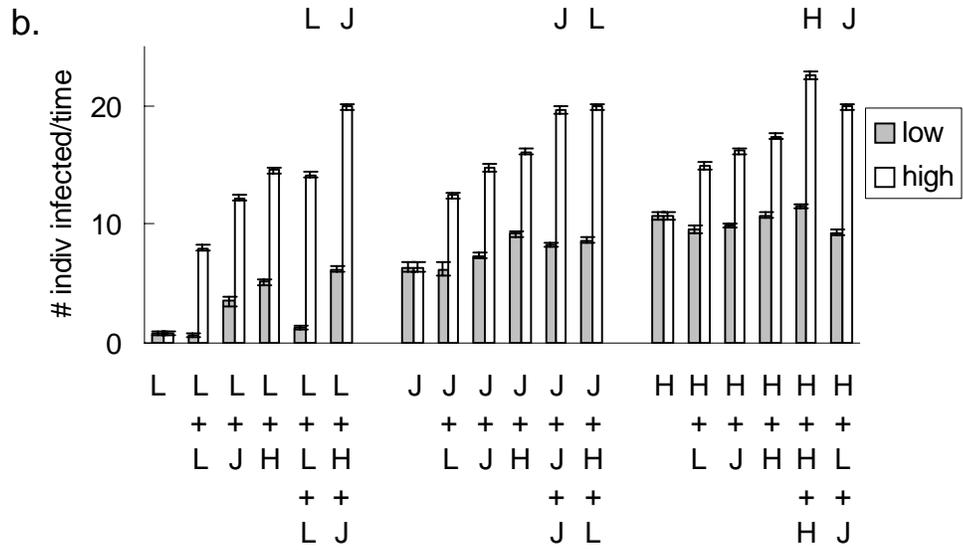
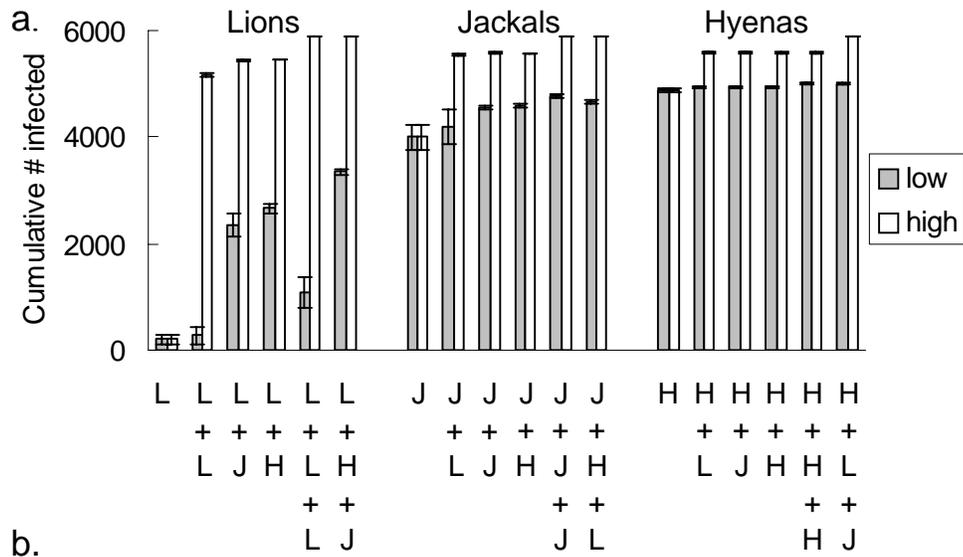


Figure 3. Cumulative number of infecteds, velocity, and percentage of simulations causing an epidemic for each combination of species. **(a)** The average cumulative number of infected individuals for each of the species listed at the top of the panel, in isolation, and combined with 1 and 2 other species where L=lion, H=hyena, J=jackal. Gray bars, low coupling; white bars, high coupling; error bars, 95% CI. **(b)** The velocity of infection (number of infections per time-step) per combination of species. **(c)** Percentage of simulations ($n = 150$) that cause an epidemic (defined as infection persisting longer than 200 time-steps).

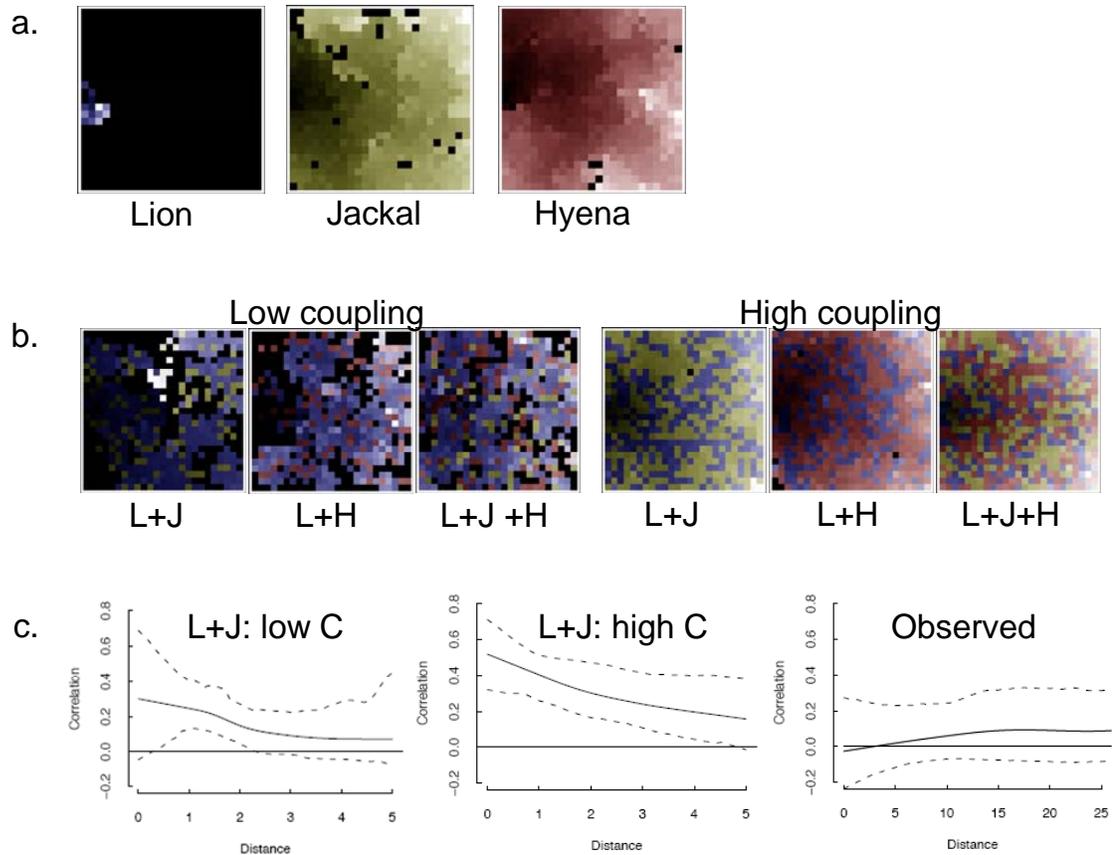


Figure 4. Spatial spread simulations and correlations. **(a)** Spatial spread of infection from a single example of a simulation in lions, jackals and hyenas, respectively; **(b)** simulated multispecies epidemics involving lions. The color of each simulated grid cell represents the source of infection in lions in a single example (blue, lion; yellow, jackal; red, hyena), and colors grade from early (dark) to late infection (light); uninfected cells are black. **(c)** Spatial correlations for simulated and observed outbreaks. For simulated epidemics, each plot shows mean estimates (solid line) and 95% bootstrap CI (dashed lines) based on 1000 randomly chosen 5x5 subgrids. For the observed epidemic, each plot shows distance (km) vs. spatial correlation for the mean estimate (solid line) and 95% bootstrap CI (dashed lines). NCF figures were similar for the other two-species combinations and the three-species scenario.

Supplementary Material

A	B	# Inf (low)	# Inf (high)	Vel (low)	Vel (high)
L	J				
H	J				
H	L				
L	L+L	X		X	
L	L+H				
L	L+J				
L	L+L+L			X	
L	L+H+J				
L+L	L+H				
L+L	L+J				
L+L	L+L+L			X	
L+L	L+H+J				
L+H	L+J		X		
L+H	L+L+L				
L+H	L+H+J				
L+J	L+H+J				
L+J	L+L+L				
L+L+L	L+H+J		X		
H	H+H			X	
H	H+L	X			
H	H+J				
H	H+H+H				
H	H+J+L				
H+H	H+L	X	X		
H+H	H+J	X	X		
H+H	H+H+H		X		
H+H	H+J+L				
H+L	H+J	X	X	X	
H+L	H+H+H		X		
H+L	H+J+L			X	
H+J	H+H+H		X		
H+J	H+J+L				
H+H+H	H+J+L	X			
J	J+J			X	
J	J+H				
J	J+L	X		X	
J	J+J+J				
J	J+H+L				
J+J	J+H	X	X		
J+J	J+L	X		X	

J + J	J+J+J				
J + J	J+H+L				
J+H	J+L	X			
J + H	J+J+J				
J+H	J+H+L	X		X	
J+L	J+J+J				
J+L	J+H+L	X			
J+J+J	J+H+L		X		X

Table S1. Pairwise comparison between means for 95% CI's. We compared the means between the simulation runs in Column A and Column B (representing comparisons between histogram bars in Figure 3a,b) using simultaneous confidence intervals with the Bonferonni correction set to 48 groupings. An “X” signifies that the means are not statistically different. “# Inf” is cumulative number of infected individuals and “Vel” is the velocity, shown for both high and low coupling. In general, means between simulations with high coupling are statistically different (with the exception of number of infections in hyenas), whereas means between velocity and cumulative number of infections with low coupling are often not statistically meaningful. Those means that are not statistically significant do not influence the overall conclusions of the paper.

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