Distributions, Drivers, and Risks of Wildlife Infectious Diseases across Africa: using geospatial analyses to elucidate disease occurrence in biodiversity hotspots

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Abstract

This dissertation focuses on wildlife infectious diseases in Africa with a goal of using geospatial analyses to better understand their distributions, drivers, and risks. Using widespread data from Simian Foamy Virus (SFV) infections in humans, I identified bushmeat hunting and proximity to active commercial logging sites as the largest drivers of crossover from non-human primates. I created a risk map for humans to compare to models of SFV, Simian Immunodeficiency Virus (SIV), and Ebola Virus Disease (EVD) occurrence in wild populations of non-human primates across the Congo Basin. A hotspot of highest predicted zoonotic crossover risk to humans appears in northern Gabon and neighboring portions of Cameroon and Republic of the Congo where disease occurrence, bushmeat hunting intensity, and logging activities are high. Efforts to reduce demand for bushmeat in urban centers along with education of the risks of bushmeat consumption may help reduce the chance of future zoonotic outbreaks.

To evaluate the roles that current and future climatic conditions may play in shaping wildlife infectious disease occurrence, I investigated SIV and SFV infections in chimpanzees. Using ensemble niche modeling techniques, I found that the occurrence of SIVs and SFVs infecting three chimpanzee subspecies across Central Africa remain divergent under both present and future climatic conditions across Central Africa. The geographic separation of these two pathogens within chimpanzee subspecies where they are both present may be explained by viral competition for resources within chimpanzee hosts, localized pockets of immunity

due to previous outbreaks, range restrictions due to the ecological niches of chimpanzee hosts, or geographic restriction of infected chimpanzees due to geographic or behavioral boundaries.

To investigate how biodiversity may drive wildlife infectious disease occurrence, I focused on EVD infecting chimpanzees and gorillas in western equatorial Africa. I tested two hypotheses: the Dilution Effect, which states that disease occurrence is inversely correlated with biodiversity, and the Amplification Effect, which states that disease occurrence is directly correlated with biodiversity. In the case of African apes, low levels of primate species richness (host taxa) and high levels of bat species richness (potential natural reservoir taxa) appear to drive EVD occurrence. Frugivorous bats are a putative natural reservoir for Ebola Viruses in Africa, and these results suggest that multiple species may act as equally competent reservoirs for the virus, where areas of high bat species richness are likely to coincide with EVD occurrence sympatric non-human primates.

Lastly, I developed two new products to further the field of distribution modeling: TERRA6-AR5 and Biomod2EZ. TERRA6-AR5 is a multi-model ensemble of current climate change model projections at a global extent including all four representative concentration pathways defined by the International Panel on Climate Change for years 2030, 2050, and 2080 at a high spatial resolution ($\sim 1km^2$). Biomod2EZ adds missing functionality in the original Biomod2 package including a report generation feature to save results in an organized fashion, export maps for use in external mapping software, and simplifying the overall modeling process with annotated scripts and tutorial datasets.

Chapter 1: Background

This dissertation addresses how different factors may contribute to the occurrence, distribution, and future of different wildlife infectious diseases across Africa. Africa is a center for biodiversity and infectious disease, but the exact mechanisms that shape their distributions are not entirely understood. Until recently, few opportunities existed to quantify contributors to disease occurrence on large scales, but with advances in distribution modeling techniques and comprehensive pathogen/disease data from diverse organisms, it is possible to predict disease occurrence and identify relationships with external forces (Roy-Dufresne et al., 2013; Harrigan et al., 2014; James et al., 2015). This dissertation investigates the relationships between wildlife infectious disease occurrence and zoonotic outbreaks into human populations, the effects of climate change on disease distributions, and the roles that biodiversity play in promoting or deterring disease occurrence using modern ecological niche modeling techniques.

Wildlife across Africa, particularly in Central Africa, are already under threat from a variety of pressures that can significantly affect their distributions and movement (Tranquilli et al., 2014). These animals often carry diseases that have potential to cause mortality and spread through communities and across species (Brearley et al., 2013). A broader understanding of the drivers that shape the occurrence of different infectious diseases in wildlife is vitally important since this region of the world is currently undergoing drastic changes. Africa's human population is expected to double to 2.4 billion by 2050 (25% of the global total), and eventually

reach 4.2 billion by 2100 (40% of the global total) (Un Desa, 2015). While human populations are expanding, a socioeconomic transition is sweeping through the region, fueled largely by foreign investment focused on the extraction and exploitation of natural resources, such as timber and minerals (Du Bois, 2014). Amidst the expansion of humans across this region, Central Africa is also home to some of the richest biodiversity on the planet, especially within the Gulf of Guinea and Congo Basin rainforests that harbor 20% of all known plant and animal species (Jones, 1994; Besselink et al., 1998; Oates et al., 2004). These ecosystems are under increasing pressure from habitat alteration, deforestation, and loss of biodiversity due to unprecedented population growth and unsustainable development (Hansen et al., 2013; Zinkina et al., 2014). Underpinning these threats is the growing certainty that climate change will radically transform the region's forests, which places at least 30% of Central Africa's plants and animals at risk of extinction (Thomas et al., 2004; Rudel, 2013).

These compounded pressures will likely affect wildlife, their habitats, and their distributions along with the infectious diseases that they carry (Jones et al., 2008). Wildlife infectious diseases can cause detrimental effects in animal hosts and are a principle concern for conservation (Leroy et al., 2004b; Bermejo et al., 2006). Understanding drivers of disease occurrence in wildlife populations is important for the future of host species and encroaching human populations. This study is aimed at understanding the contributions of three potential drivers of wildlife infectious disease occurrence in Africa: (*i*) anthropogenic pressure and zoonotic crossover, (*ii*) present climate and climate change, and (*iii*) biodiversity. These three proposed

causative agents were used to test predictions based on existing theories relating to infectious disease occurrence in wildlife populations using a combination of remote sensing, geospatial processing, climate modeling, and ensemble distribution modeling.

Ecological niche theory

Over time, different concepts of the ecological niche have been proposed by a number of researchers, dating back to the early 1900s (Levin et al., 2009). The "Grinellian niche concept" defined a niche as the habitat in which a species lives and its behavioral adaptations that allow it to survive and reproduce successfully (Grinnell, 1917). In this study, Grinnell focused on the California Thrasher and noted how it utilized its habitat effectively for food, reproduction, and avoiding predators. The "Eltonian niche concept" defined a niche as a species' place in the biotic environment and its relationships with food sources and predators (Elton, 1927). According to this concept, a herbivore's niche may be defined by the availability of plants that it eats, and may overlap with a carnivore's niche that preys on the herbivore. The "Hutchinsonian niche concept", which appears to be the most accepted definition today, defined a niche as the combination of necessary environmental conditions and resources for a species to survive (Hutchinson, 1957). This niche is known as an "n-dimensional hypervolume" which is a space defined by *n* dimensions of variables that are biologically important to the target taxa, each of which falls on its own axis (Blonder et al., 2014). The hypervolume exists as a set of points that fall within the bounds of the n-dimensions, where each point defines the

requirements for the survival of the study taxa. This concept of ecological niches also leaves the opportunity for empty niches to arise from extinction of migration, which can be filled by other taxa. The ecological niche of a given species can be further subdivided into at least two categories: the fundamental niche and the realized niche (Peterson, 2006b; Holt, 2009). A fundamental niche refers to the range of conditions that a species could theoretically inhabit, and successfully survive and reproduce in the absence of competition with other species. A species' realized niche is a subset of its fundamental niche, and is defined as the actual space inhabited by a species in reality, the range of environmental conditions that occur in this space, and the resources the species can access as a result of limiting pressures exerted by other species (Grinnell, 1917; Hutchinson, 1957; Whittaker et al., 1973).

Modeling ecological niches and species' distributions

Species distribution models (SDMs), ecological niche models (ENMs), and predictive habitat models are different terms used to describe the process of predicting the occurrence of a species or population as a function of underlying environmental measures (Hirzel et al., 2008; Peterson et al., 2011). Their uses span a variety of disciplines including, but not limited to, conservation, studies of invasive species, biodiversity assessments, and predicting disease occurrence (Peterson et al., 2001; Peterson, 2003; Wiens et al., 2005; Domínguez-Domínguez et al., 2006; Peterson, 2006c; Peterson, 2006b; Elith et al., 2009). Predictive models offer an affordable way to make large-scale predictions using widely available spatial data without the need to systematically sample entire regions on the ground. Their

methods can range from relatively simple to more complex, but all aim to identify a relationship between known occurrences with underlying environmental measures to be able to predict the probability of occurrence in unsampled regions (Austin, 2007).

The earliest attempt at ecological niche modeling involved niche-based predictions of crop species in Australia using a mechanistic approach, with details of plant physiology and resource requirements, unlike the largely correlative approaches that are most popular today (Nix et al., 1977). One of the first studies using a correlative approach was published a few years later, and involved using eight "ecoclimatic" factors to quantify the environmental requirements of over 80 plant species (Box, 1981). These niche requirements were then used to predict the occurrence of these plants at locations across the world based on a coarse grid dataset of the same ecoclimatic factors with a spatial resolution of over 1000km^2. In the mid-1980s into the 1990s, many new modeling approaches and tools were proposed and released, taking advantage of more powerful statistical analyses available from advances in computer science (Guisan et al., 2005).

BIOCLIM was the first widely accessible ENM technique used to predict the "climate profile" of a species (Busby, 1991). BIOCLIM is a type of climate envelope model that uses details of the underlying climate to create minimum and maximum bounds around a species' occurrence to define the "climate envelope" in which the species can be found (Watling et al., 2013). BIOCLIM can be used to interpolate up to 35 climatic measures across a study region that it then uses to define the bounds of a

species' climate envelope, but it has been shown that increasing the number of predicting variables used in climate envelope models results in more restricted niches due to overfitting (Beaumont et al., 2005). Other climate envelope models that came later on include ANUCLIM (Busby, 1991), DOMAIN (Carpenter et al., 1993), and DIVA-GIS (Hijmans et al., 2001).

Random Forests (Liaw et al., 2002) is a presence-absence model that is useful when reliable absence localities are available. In general, presence-absence models require the input of known locations where a species is found and locations where it is absent (Peterson et al., 2011). The Random Forests approach implements a regression tree method that searches for the best split at each node from a subset of the environmental predicting variables. Trees are grown without pruning, creating a "forest" of diverse trees. For each tree, a subset of the presence data is withheld and run through the tree in order to calculate the number of times that they are classified incorrectly as a statistical measurement of the classification error of each model (Breiman, 2001; Liaw et al., 2002). Random Forests has also been found to rarely over-fit models, and is touted for successfully managing large sets of environmental variables while overcoming noise caused by extraneous factors that do not contribute to the overall model (Cutler et al., 2007). Models that are over-fit describe noise in the data as opposed to the relationship between the environmental variables that drive the signal (Breiman, 2001).

In some cases, accurate absence localities can be difficult to obtain for study taxa with large home ranges or those that undergo seasonal migrations. Presence-only

models do not require absence localities, and instead use 'pseudo-absence' points that are created during the modeling process. Maxent (Phillips et al., 2006) is a presence-only ecological niche modeling software package that is commonly used in literature today, and has been shown to outperform other presence-only models such as GARP (Genetic Algorithm of Rule-set Prediction) (Elith et al., 2006). Absence data can often be unreliable, especially when studying highly mobile or elusive taxa with large home ranges, making presence only models a better choice in some situations (Sesink Clee et al., 2015). ENMs constructed using Maxent begin with known presence localities for the population in question, along with pseudo-absences plotted across the study region. Starting with the assumption of a uniform distribution of the population in question, Maxent implements the principle of maximum entropy (deviating from a normal distribution only enough to meet the niche criteria) while including many possible combinations of environmental variables to create a probability distribution of habitat suitability across environmental space (Phillips et al., 2006; Elith et al., 2011). The widespread use of Maxent today may be due to its reported performance strength, simple user interface, detailed documentation, lack of need for true absence data, and its strength in creating models using small presence datasets (Elith et al., 2006).

From a user's perspective, one potential issue is the plethora of modeling techniques available to choose from today. It can be quite daunting to select only one model to use, while being able to properly justify that it is better than other available options. Different models can produce varied results since they use different mathematical operations during the modeling process (Araújo et al., 2007). One way to get a

better understanding for how different models fit a given dataset is through ensemble forecasting or ensemble modeling (Araújo et al., 2007). In general, ensemble modeling consists of performing multiple runs across a set of initial conditions, models, and parameters, creating a number of possible combinations with different results that can be summarized into a single consensus model (Figure 1) (Bates et al., 1969). In terms of species distribution modeling, the Biomod2 package (Thuiller et al., 2013) for the R framework (R Development Core Team, 2016) can be used to run up to11 different distribution models including regression and climate envelope based techniques on a given dataset through ensemble forecasting.

Biomod2 can be used to calculate the model accuracy, or predictive power, for each distribution model using the following test statistics: area under the relative operating characteristic curve (AUC: Hanley et al., 1982), Cohen's K (KAPPA: Monserud et al., 1992), and the true skill statistic (TSS: Allouche et al., 2006). From this information, one could select the model with the best overall "fit" or, alternatively, use the model accuracy values as a weighting metric to create and project an ensemble model (Thuiller et al., 2009; Thuiller et al., 2013). This method of creating an ensemble model from up to 11 different individual models helps to alleviate potential bias that may occur when using one model on its own.

All of these different methods for SDMs, ENMs, etc., aim to predict the geographic distribution and niche requirements of taxa by identifying relationships with underlying environmental factors at known presence and absence localities, and

have a wide variety of different applications. In recent years, the frequency of ENM use in publications, government, and conservation has increased dramatically (Vaz et al., 2015). This may be due to the combination of advances in modeling techniques along with the ever-increasing availability of species occurrence and remote sensing data (Peterson, 2006c). The availability of organized museum collections with known geographic origins is incredibly useful for researchers that are displaced from their study region for one reason or another. New processed geospatial data are also regularly released for public access at large spatial scales. These data have aided researchers, conservationists, and governments alike to predict the distributions of taxa and spur forward policy changes and related research (Araújo et al., 2006; Pearce et al., 2006; Hannah et al., 2007).

Predicting disease occurrence using distribution models

Presence of infectious diseases in wildlife can be difficult to predict due to the combined interactions between hosts, vectors, and reservoirs, each of which can have their own habitat requirements, dispersal routes, and methods of pathogen transmission (Keeling et al., 2008). ENM techniques are useful in estimating the probability of species presence and have been applied to the study of disease biogeography by identifying correlative relationships between known locations of disease presence within wildlife populations and underlying environmental factors that may play a role in shaping their distributions (Holt et al., 2009; Alexander et al., 2012; Flory et al., 2012). Unlike mechanistic models that require detailed knowledge of all interactions throughout the lifecycle of a disease in wildlife

populations, distribution modeling techniques are often correlative and have the potential to reveal interesting trends in disease distributions even when mechanisms of their transmission are unclear (Peterson, 2006a; Peterson et al., 2015). Some important phenomena of wildlife infectious diseases include zoonotic disease spread, the effects of climate change on wildlife infectious disease distributions, and the effects of biodiversity on wildlife infectious disease occurrence.

Zoonoses are disease-causing pathogens that have the potential to cross between wildlife and humans (Acha et al., 1987). More than 70% of emerging infectious diseases infecting humans are zoonotic, including Lyme disease, Tuberculosis, Malaria, Ebola, Rabies, and many more that have had opportunities to cross over to humans from increased contact with wildlife and via effective vectors (Jones et al., 2008; Cutler et al., 2010). A number of recent studies have focused on modeling diseases that can infect humans by taking advantage of recent advancements in ecological niche modeling techniques and the surge of available remotely sensed environmental, topographic, and anthropogenic data (Peterson et al., 2002; Peterson, 2006b; Levine et al., 2007; Mak et al., 2010). By using georeferenced samples from humans infected with diseases that are associated with wildlife, ENMs can identify ecological drivers of disease crossover, which can be used to develop mitigation measures aiming to reduce the chance of future crossover events.

Climate change has the potential to significantly affect wildlife distributions around world, along with the occurrence of wildlife infectious diseases due to their reliance

on host taxa. Diseases with lifecycles involving multiple hosts or reservoir taxa may be greatly affected by the impending increases in temperature and precipitation. For example, gradual warming in North America has already assisted tick populations carrying Lyme disease to expand northward into parts of Canada that were previously too cold for them to survive (Ogden et al., 2006). Malaria, driven by mosquito vectors, is also predicted to expand under climate change and spread into previously uninfected regions and at higher altitudes (Collier et al., 2008). Shifting distributions of wildlife that are tracking their ideal ecological niches in order to survive will invariably alter the distribution of diseases that they carry.

The relationship between biodiversity and the presence of wildlife pathogens/disease and are not well understood, but there are two competing mechanisms have been proposed to explain the relationship: the Dilution Effect and the Amplification Effect (Jones et al., 2008). The Dilution Effect hypothesis proposes that an inverse relationship exists between wildlife infectious diseases and biodiversity (Ostfeld et al., 2000). This relationship was first identified with Lyme disease in North America, where high biodiversity decreases the proportion of highly competent hosts to less competent hosts, causing a disruption in disease spread that could lead to reduced disease presence and transmission (Ostfeld et al., 2000; Keesing et al., 2006). The alternative hypothesis, known as the Amplification Effect, proposes that a direct relationship exists between wildlife infectious diseases and biodiversity (Lafferty et al., 1999; Randolph et al., 2012). In this case, increased biodiversity offers more host taxa that pathogens may be able to cross between, causing an overall increase in disease presence and transmission.

Wildlife infectious diseases are often complex, with many different factors contributing to their successful transmission and proliferation in host populations (Tompkins et al., 2011). Disease transmission can require optimal conditions in the environment, reservoir taxa, vectors, final hosts, and interactions between all of these. It is predicted that large-scale pressures, such as climate change and human expansion, will affect the distributions and presence of wildlife, which may also affect the distributions of diseases and the pathogens that cause them (Jones et al., 2008). Distribution models are useful tools to predict the occurrence of diseases in wildlife based on underlying variables that can vary from measures of climate and habitat assemblage to occurrence of potential hosts/vectors and features of the landscape the assist pathogen transmission such as bodies of water. These models can be used to target specific questions and hypotheses pertaining to disease occurrence in wildlife without the need for complete understanding of all contributing or promoting factors (Mills et al., 2010; Alexander et al., 2012).

Biodiversity of the study area

Biodiversity hotspots are regions of the world that have been designated as conservation priorities because they house an abundance of endemic, threatened, and/or rare species that are under threat of extinction due to many factors, including habitat loss, agricultural encroachment, hunting, and other impacts of human populations (Myers, 1988; Myers, 1990; Reid, 1998; Ginsberg, 1999; Myers et al., 2000; Mittermeier et al., 2011). The 25 biodiversity hotspots delineated by Conservation International (Myers et al., 2000; Mittermeier et al., 2011) contain

44% of all plant species and 35% of all vertebrate species worldwide (Figure 2). While it appears that these ecologically rich regions are quite large, they only effectively cover 1.4% of the Earth's surface today, while their primary vegetation once covered over 11% (Mittermeier et al., 2011). The recognized biodiversity hotspots across Central Africa encompass over 1.5 million km^2 of mostly dense forest habitat, of which less than 40,000 km^2 lie within protected areas (Myers et al., 2000).

A number of these biodiversity hotspots are located in Africa, specifically in the equatorial region of the continent (Figure 2). The Guinean Forests of West Africa are divided into two forest blocks, the Upper and Lower Guinean Rainforests, both of which are recognized as biodiversity hotpots (Figure 3). The Upper Guinean Forests extend along the coast from Guinea and Sierra Leone in the west, to the Dahomey Gap in Togo and Benin in the east. As the forests extend eastward they thin out towards the Dahomey Gap, which is a wide band of savannah habitat that geographically separates the Upper from the Lower Guinean Forests. The Lower Guinean Forests extend from the eastern edge of the Dahomey Gap in Nigeria along the coast into central Cameroon, where it forms an ecotone with Congolian rainforests from the south. The other biodiversity hotspot in this region of Africa, the Afromontane Forests, lies along the Albertine Rift, which is a biologically diverse region known to house over 1200 endemic plant and animal taxa (Plumptre et al., 2007). Although not identified as a biodiversity hotspot by Conservation International (Myers et al., 2000; Mittermeier et al., 2011), the Congo Basin supports a diverse set of both plant and animal taxa. Located between the Guinean Forests

and the Eastern Afromontane Forests, the Congo Basin is largely covered in dense forests with regions of forest-savannah mosaics in Central African Republic (Figure 3) (Besselink et al., 1998). Although there has been a great loss in forest cover, the remaining lowland forests of the Congo Basin hold a diverse array of plant, bird, mammal, reptile, and amphibian taxa (Doumenge, 1990; Besselink et al., 1998).

Wildlife Infectious Diseases included in this study

Infectious diseases in wildlife can be transmitted by a number of different mechanisms, including direct contact between infected individuals, via vectors passing between species, and even exist on their own in the environment before infecting a host (Cole et al., 1999). The specific biology of an infectious disease can be completely driven or shaped by the mode of transmission it utilizes. Vector-borne diseases or those with natural reservoirs are often complex in nature and require detailed knowledge of the organisms involved in each step of the pathogen's life cycle. Direct contact diseases are simpler to comprehend, as they usually involve a pathogen passing straight from one individual to another.

Infectious diseases included in this study were chosen because (*i*) they infect a wide range of taxa with varying modes of transmission which will contribute to an overarching understanding of the distributions of wildlife infectious disease presence, (*ii*) samples from wild populations of infected taxa have been found across the continent and are available from a number of institutions, (*iii*) the implications of understanding infectious diseases that are known to be harmful to wildlife is of

the utmost concern for conservation, and (*iv*) some of these diseases are zoonotic and detailed analysis of these may shed light on recent outbreaks in humans.

Ebola Virus

Ebola virus (EBOV), of which there was a recent outbreak in West Africa, causes Ebola hemorrhagic fever (EHF), also known as Ebola Virus Disease (EVD), with symptoms in humans and other mammals including vomiting, diarrhea, fever, and internal/external bleeding (Gire et al., 2014). Primates infected with EVD, including Western Lowland Gorillas and chimpanzees, have very low chances of survival, with mortality rates of infected individuals reaching above 90% (Huijbregts et al., 2003; Leroy et al., 2004a; Rouquet et al., 2005; Bermejo et al., 2006; Lahm et al., 2007). Infections also cause high mortality rates in monkeys, antelope, and wild pig species across Africa (Formenty et al., 1999; Huijbregts et al., 2003; Leroy et al., 2004b; Bermejo et al., 2006; Lahm et al., 2007; Ryan et al., 2011). Fruit bats may act as a natural reservoir of the Ebola virus (Leroy et al., 2005) that maintain and transmit it to other animals and humans through contact with bodily fluids, such as saliva on partially eaten fruit and blood when hunting bats for bushmeat (Leendertz et al., 2016a). Once infected, EBOV invades a variety of cells (including the endothelial cells of blood vessels, macrophages, monocytes, and liver cells) and replicates within cell membranes, which results in the breakdown of endothelial cells leading to hemorrhaging (Leroy et al., 2007). EBOV can then interfere with the signaling of some white blood cells, which allow it to travel throughout the body to different tissues and organs to infect. Infected animals can

transmit EBOV to other individuals by direct contact with bodily fluids including blood, saliva, sweat, vomit, urine feces, breast milk, and semen (Center for Disease Control (CDC), 2014).

Simian Immunodeficiency Virus

Simian Immunodeficiency Viruses (SIVs) are retroviruses that infect 45 species of non-human primates and are transmitted between individuals by direct contact with bodily fluids, including saliva, semen, blood, and urine (Peeters et al., 2002; Locatelli et al., 2014) and there is evidence that it may transmit vertically from mother to child, potentially via milk (Apetrei et al., 2004). Although SIV infections in primates sometimes appear to be non-pathogenic, there have been cases of apes dying from what appear to be AIDS-like symptoms (Keele et al., 2009). In infected non-human primates, SIVs infect CD4+ T cells, which play important roles in immune response, including the activation of cytotoxic T cells and macrophages (Keele et al., 2009). Infected CD4+ cells undergo apoptosis, leading to progressive deterioration of immune system response by loss of these lymphocytes over time, resulting in an immunodeficiency syndrome (Novembre et al., 1997). Strains of SIV from chimpanzees (*Pan troglodytes*), gorillas (*Gorilla gorilla*), and sooty mangabeys (*Cercocebus atys*) are also believed to be the progenitors of HIV-1 and HIV-2 in humans (Figure 4) (Locatelli et al., 2014). Spillover into human populations most likely occurred by humans coming into contact with infected primate blood or tissue while handling carcasses hunted for bushmeat (Van Heuverswyn et al., 2007b).

Simian Foamy Virus

Simian foamy viruses (SFVs) are widespread retroviruses that have been found infecting all non-human primate species across Africa and are believed to transmit between individuals via saliva (Linial, 1999; Mouinga-Ondémé et al., 2012; Stenbak et al., 2014). Like other retroviruses, SFVs integrate their genetic material into the host genome and rely on the host cell factors for viral gene expression (Murray et al., 2006). SFVs have an ancient origin, and appear to have been infecting primates for up to 30 million years based on estimates of substitution rates of viral genome sequences. Additionally, host/virus phylogenies suggest that SFVs co-speciated with their non-human primate hosts (Verschoor et al., 2004; Switzer et al., 2011). In infected individuals, SFVs target lymphocytes involved in immune response and trigger the fusion of cells into multi-nucleated syncytia that have a foam-like appearance, but this does not appear to be directly harmful to their hosts (Linial, 2000; Meiering et al., 2001; Murray et al., 2006). Although this pathogen is not known to cause adverse symptoms in primates, there is evidence that pre-existing SFV infections can increase an individual's susceptibility to subsequent, more detrimental, infections and diseases (Choudhary et al., 2013). Its high prevalence in both captive and wild non-human primates (Meiering et al., 2001; Murray et al., 2006; Stenbak et al., 2014) has contributed to increased transmissions to humans, making it a concern for public health since its affects are not fully understood (Liu et al., 2008; Betsem et al., 2011; Huang et al., 2012; Mouinga-Ondémé et al., 2012).

Chytridiomycosis

The fungus *Batrachochytrium dendrobatidis* causes the disease chytridiomycosis (also known as chytrid) in amphibians. *B. dendrobatidis* is a water-borne pathogen that disperses via motile zoospores that use flagella to move through aquatic environments searching for host amphibians to settle in their epidermis (Longcore et al., 1999). Zoospores then develop into sporangia that produce additional zoospores, which can disperse through water or reinfect the same host individual (Berger et al., 2005). Symptoms of chytrid in amphibians include increased skin shedding, epidermal lesions, and the thickening of outer epidermal layers (Berger et al., 2005). Although visible signs often remain unseen because they may only appear 15 days post-exposure, they also include abnormal posture, spasms and extensions of hind limbs, and failure to seek shelter and flee from danger (Berger et al., 1998; Carey et al., 2006). The thickening and increased sloughing of skin appears to be the major cause of mortality as it severely limits individuals' ability to release toxins, absorb nutrients, and respire (Pessier, 2002). This non-hyphal fungus has rapidly become the leading cause of amphibian declines in some species, while other species appear to be less susceptible to infections (Bosch et al., 2001; Collins et al., 2003; Daszak et al., 2003; Fisher et al., 2009; Lötters et al., 2009).

Dissertation Structure

This dissertation is organized into five chapters that address how different factors may contribute to the occurrence, distribution, and future of different

wildlife infectious diseases across Africa. In Chapter Two, I investigate how anthropogenic pressures may contribute to zoonotic disease crossover from wildlife to humans across Central Africa including Ebola, Simian Foamy Virus, and Simian Immunodeficiency Virus. The aim of this chapter was to use human actions that can increase disease crossover risk from wildlife to identify regions where crossover events may be more likely to occur. A core region of high crossover risk was identified in northern Gabon where disease presence and human pressures such as bushmeat hunting are high. In Chapter Three, I expanded on a previous study based only in Cameroon that identified a potential inverse relationship between the occurrence of Simian Foamy Virus and Simian Immunodeficiency Virus infected chimpanzees to include the full ranges of the Nigeria-Cameroon Chimpanzee (*Pan troglodytes ellioti*), the Central Chimpanzee (*P. t. troglodytes*), and the Eastern Chimpanzee (*P. t. schweinfurthii*). Additionally, occurrence models were projected under climate change representative concentration pathways to test how pathogen distributions may shift over time. These models show that an overall inverse relationship between SFV and SIV persists across *P. t. troglodytes* and *P. t. schweinfurthii* under present and future climate change. In Chapter Four, I investigated the role that community and host biodiversity may play in Ebola Virus Disease occurrence in non-human primates. Here, I tested two competing hypotheses: the Dilution Effect hypothesis, which recognizes an inverse relationship between disease presence and biodiversity, and the Amplification Effect hypothesis, which recognizes a direct relationship between disease presence and biodiversity (Jones et al., 2008). Distribution models showed a strong association between Ebola

Virus Disease presence and regions of low primate species richness (host taxa) and high bat species richness (potential natural reservoir taxa), showing support for the Dilution Effect in terms of hosts, and the Amplification Effect in terms of natural reservoirs. In Chapter Five, I present a new multi-model ensemble called TERRA6-AR5 that includes the current 4 climate change representative pathways (Ipcc - Intergovernmental Panel on Climate Change, 2014) for years 2030, 2050, and 2080, encompassing 20 different global climate models, produced at a high spatial resolution (about $1km^2$) at a global extent. In this chapter, I provide an example application of the dataset by projected ecological niche models for fungal chytridiomycosis infections in amphibians across all of Africa, showing distribution shifts to higher elevations and into regions where it is currently absent. Finally, in Chapter Six, I present an R-script suite that I created called Biomod2EZ that expands on the ensemble model package Biomod2 by adding reporting capability to streamline multiple iterations of the model and automatically generate clear reports and output files for use in external mapping software. Included in this script suite, I annotated all code and wrote a tutorial with a sample dataset, so that users that are inexperienced with using R for spatial modeling can understand each step of the process including how to create input files, modify model parameters for best fit, and modify the baseline code to best suite their datasets.

Figures

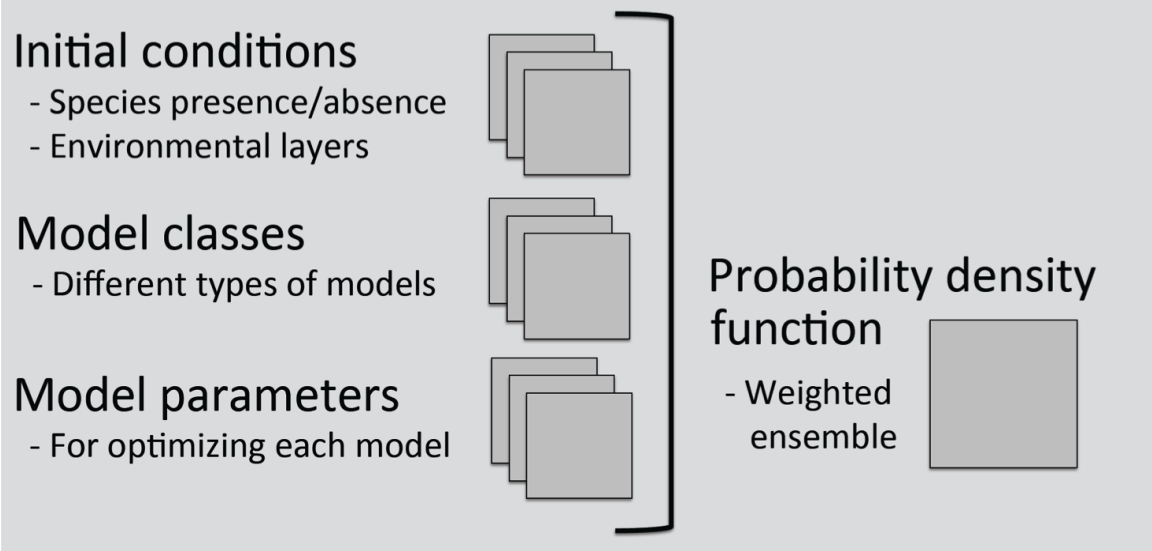

Figure 1. Ensemble modeling technique overview (Adapted from Thuiller et al., 2009). The general process of ensemble modeling involves creating a set of predictions using the same initial conditions with a variety of model classes and parameterizations. Individual models can be combined to create a single ensemble model that represents the range of outcomes predicted by individual contributing predictions.

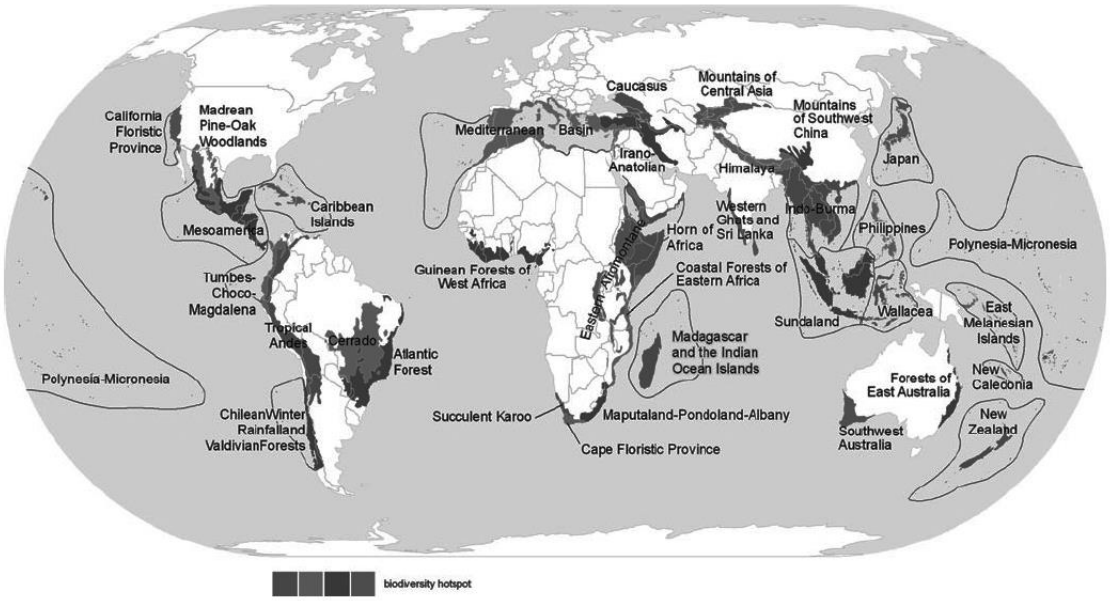

Figure 2. Map of global biodiversity hotspots from Conservation International (Mittermeier et al., 2011). In Central Africa, identified biodiversity hotspots include the Guinean Forests of West Africa and the Eastern Afromontane.

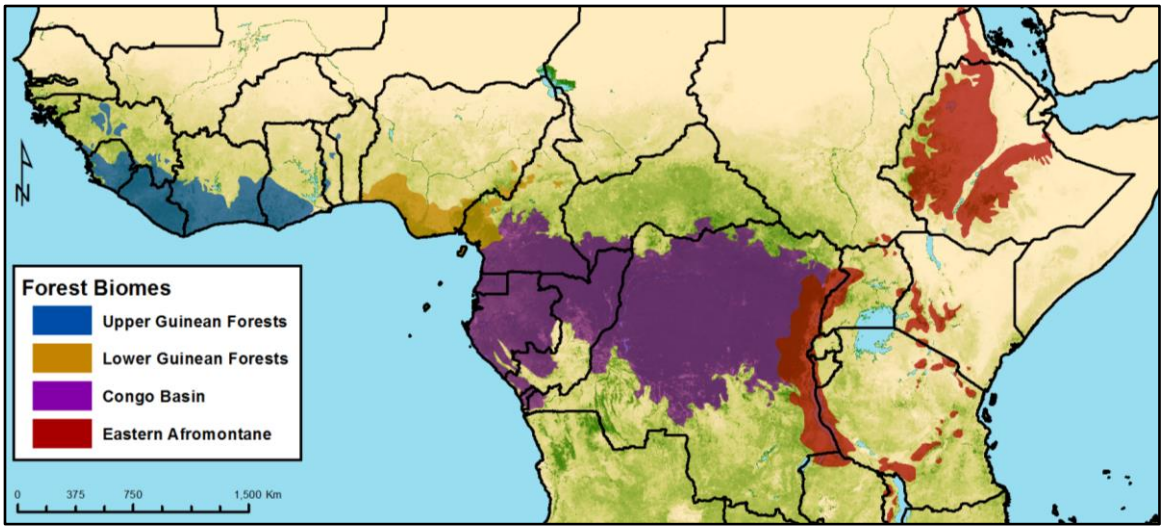

Figure 3. Map of Central Africa's major forest biomes: the Upper and Lower Guinean Forests, Congo Basin, and Eastern Afromontane Forests.

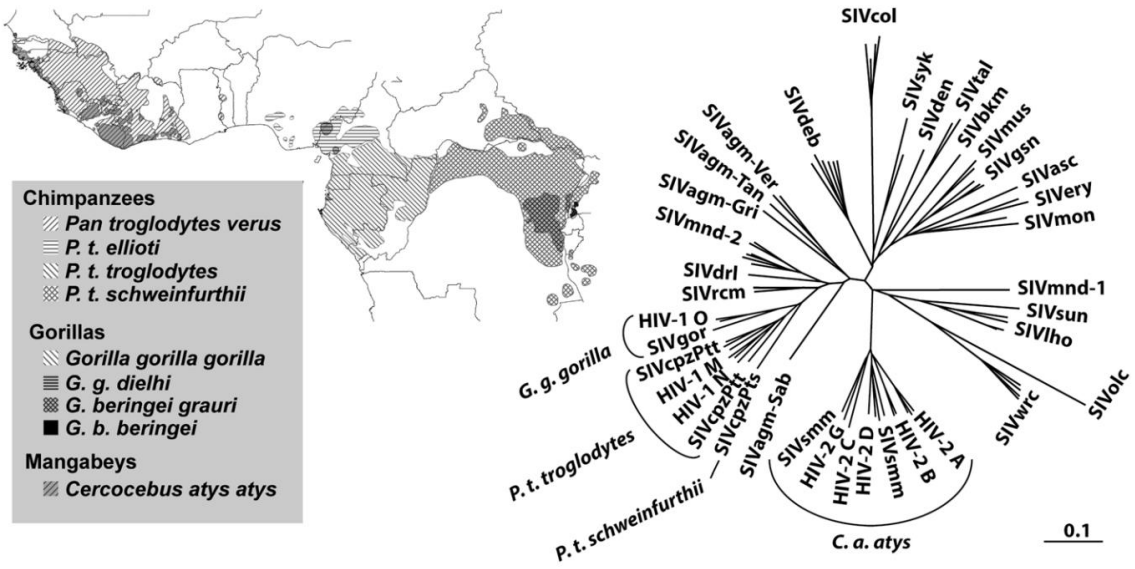

Figure 4. Distributions and phylogeny of SIVs infecting non-human primates. Geographical ranges of sooty mangabeys, chimpanzees, and gorillas infected with SIV and phylogenetic tree from different SIVs infecting nonhuman primates and HIVs infecting humans (Locatelli et al., 2014).

Chapter 2: Assessing the drivers and transmission risk of wildlife infectious diseases from non-human primates to humans in the Congo Basin

Abstract

The majority of emerging infectious diseases in humans today have origins in wildlife. Over time, from increased contact with domesticated and wild animals, transmissible pathogens have ample opportunities to cross from one species to another. Across Central Africa, where human population increase and expansion is ongoing, local people encroach deep into previously uninhabited forests where a plethora of animals remain. In this region, hunting of wildlife for meat has expanded from a means of nutrition for individual families and villages to a thriving business with bushmeat markets in every major city. Commercial bushmeat hunting is fueled by high demand from urban centers and sustained by easy access to forests from new roads and trails, many of which are built by the rapidly growing commercial logging industry.

In this study, I identified anthropogenic drivers of zoonotic disease crossover from non-humans primates to humans across the Congo Basin. I then created distribution models using environmental predicting factors for Ebola Viruses, Simian Foamy Viruses, and Simian Immunodeficiency Viruses infecting wild chimpanzee (*Pan* spp.), gorilla (*Gorilla* spp.), and mandrill (*Mandrillus sphinx*) populations in order to locate centers of disease presence in non-human primates across the Congo Basin. Finally, a wildlife infectious disease hotspot map was created from the overlap of individual disease distribution models and was assessed

for the intensity of anthropogenic activities that were found to promote crossover of zoonoses from non-humans primates.

Using the widespread, and easily transmissible Simian Foamy Virus as an example, I determined that areas of high bushmeat hunting with close proximity to active logging sites and roads are most highly correlated with zoonotic disease transmission events to humans. I also identified an overall non-human primate zoonotic hotspot in northern Gabon and neighboring parts of Cameroon and Republic of the Congo where co-occurrence of Ebola, Simian Immunodeficiency Virus, and Simian Foamy Virus is predicted. High levels of bushmeat hunting and logging also occur in this region, making it an area of high-risk for disease crossover. Increased enforcement of protected areas where there appear to be high levels of hunting in this region, combined with initiatives to reduce urban demand for bushmeat, may jointly help reduce contact between humans and disease-carrying non-human primates.

Introduction

Human footprint expansion and population growth has continued to increase steadily over time, encroaching on wildlife and previously untouched parts of the world (Mckee et al., 2004). As human populations expand geographically, we create dense road networks that divide animal populations into isolated groups, alter habitats by deforestation for logging and agriculture, and hunt wildlife for sustenance and sport (Defries et al., 2010). These activities and many more that humans impose on the environment bring local people in close contact with wildlife

and, in turn, wildlife infectious diseases (Daszak et al., 2001; Patz et al., 2004; Woodroffe et al., 2011; Morand et al., 2014). More than 70% of emerging infectious diseases in humans are zoonotic in origin, meaning that they have crossed species boundaries from wildlife to humans, including Lyme disease, Tuberculosis, Malaria, Ebola, Rabies, and others that are well-suited for transmission to humans (Jones et al., 2008; Cutler et al., 2010). Zoonoses in the tropics are more likely to involve animal reservoirs, especially in non-human primates and represent a weak barrier of transmission to humans because they are so closely related, than in temperate regions, potentially because of the lack of closely related taxa (Wolfe et al., 2012). In addition, increased human contact with wildlife in the tropics from hunting, logging, and agriculture further increases the chances of cross-species transmission events because many zoonoses in these regions originate from mammals, such as primates (Jones et al., 2008).

Recent zoonotic outbreaks in humans across west Central Africa

Central Africa has been the center for a number of different zoonotic outbreaks in humans, including most recently, Ebola, where repeated outbreaks have occurred in Democratic Republic of the Congo, Guinea, Liberia, Sierra Leone, and Nigeria (Davis et al., 2001a). Since the early 2000s, there have been multiple Ebola outbreaks in Central and West Africa that collectively have infected and killed more humans than any previous Ebola outbreak, with more than 28,000 human cases in all (World Health Organization, 2016). Although many of these outbreaks were heavily publicized by news agencies, one outbreak in Gabon and Republic of

the Congo had far fewer headlines: the outbreak of Ebola in gorillas and chimpanzees (Bermejo et al., 2006). Since around 2000, Ebola outbreaks in humans in Gabon and Republic of the Congo have been coupled with the discovery of chimpanzee and gorilla carcasses infected with Ebola in nearby forests (Huijbregts et al., 2003). Researchers studying habituated gorillas in this region observed a north-south spread of Ebola, estimated to have occurred from a combination of mass spillover from a reservoir host and transmission between different gorilla groups, causing death in over 95% of the population (Bermejo et al., 2006). In a neighboring forest block, upwards of 98% of chimpanzees and gorillas were also infected with the Ebola virus, which in recent years, led to the loss of over 4,000 individuals in total (Huijbregts et al., 2003).

In this region of Central Africa, there are several other zoonotic infections that occur in primates and have the potential to spread to humans, such as Simian Foamy Viruses (SFV), which are common in primate species across Africa (Murray et al., 2006). On a cellular level, SFVs target cells involved in immune response such as helper T cells and macrophages (Clapham et al., 1991) and form syncytia by fusion of cells into a multi-nucleated mass (Linial, 1999). Many studies have identified SFV infections in humans that work closely with non-human primates in sanctuaries and in bushmeat hunters that regularly hunt and butcher non-human primates (Switzer et al., 2004; Wolfe et al., 2004a; Wolfe et al., 2005b; Calattini et al., 2007; Huang et al., 2012; Mouinga-Ondémé et al., 2012), but it appears that all human infections of these foamy viruses are zoonotic in origin and cannot transmit between humans (Betsem et al., 2011; Stenbak et al., 2014).

This study region also encompasses the epicenter of the origins of Human Immunodeficiency Virus in southern Cameroon (Santiago et al., 2002; Keele et al., 2006). Strains of the Simian Immunodeficiency Virus likely spread from the Central African subspecies of chimpanzees (*Pan troglodytes troglodytes*) to humans becoming HIV-1 types M and N, from gorillas (*Gorilla gorilla gorilla*) to humans becoming HIV-1 type O (Gao et al., 1999; Keele et al., 2006; Van Heuverswyn et al., 2007b; Locatelli et al., 2014; D'arc et al., 2015), and from sooty mangabeys (*Cercocebus atys*) in northwest Africa to humans that developed into HIV-2 types A, B, C, and D (Silvestri et al., 2005; Wertheim et al., 2009). These cross-species transmission events are hypothesized to have occurred by blood contact with open wounds in humans while they were handling and butchering the carcasses of infected primates that were hunted (Linial, 2000; Murray et al., 2006; Mouinga-Ondémé et al., 2011). Less is known the transmission of these viruses between ape species, and whether gorillas initially acquired SIVs from chimpanzees, but evidence suggests that this is unlikely due to differentiation between viral strains and limited interaction between these taxa in the wild (Van Heuverswyn et al., 2006; Neel et al., 2009).

In this study, I aimed to assess zoonotic crossover risk from wild non-human primates to humans using a three step approach: (1) determine anthropogenic encroachment pressures that are most closely associated with recent zoonotic crossover of a widespread pathogen from non-human primates to humans across the Congo Basin, (2) create distribution models for a set of zoonotic diseases in non-human primate populations, and (3) combine disease distribution models to create

a zoonotic disease hotspot map and analyze its overlap with identified human pressures that may increase zoonotic crossover risk. Using this approach, I identified encroachment pressures that increase the risk of zoonotic disease crossover from non-human primates to humans, and highlighted areas within the Congo Basin with the high zoonotic crossover risk. These areas of high zoonotic crossover risk represent regions where presence of zoonoses in wild non-human primates coincide with anthropogenic pressures that may increase risk of zoonotic crossover.

Methods

Identifying anthropogenic activities that increase disease crossover risk from non-human primates

SFVs are widespread and can be found in all species of non-human primates across Africa (Murray et al., 2006). The combination of its large geographic distribution and variety of non-human primate hosts contribute to its high transmissibility to humans (Switzer et al., 2004; Morozov et al., 2009; Betsem et al., 2011). SFVs can only be contracted in humans from direct contact with non-human primates and are unable to pass horizontally or vertically between infected individuals (Linial, 2000). SFV infections also do not appear to cause disease or detrimental effects in humans (Richard et al., 2015). For these reasons, SFV is a useful zoonotic infection to determine how and which human encroachment activities represent the greatest risk for zoonotic transmission from non-human primates. Several studies throughout the Congo Basin have reported the occurrence

of SFV infections in local people where the highest infection rates occur in those involved in the hunting, preparation, sale, and consumption of non-human primates (Goepfert et al., 1996; Heneine et al., 1998; Wolfe et al., 2004a; Calattini et al., 2007; Switzer et al., 2008; Betsem et al., 2011; Mouinga-Ondémé et al., 2011; Switzer et al., 2011; Mouinga-Ondémé et al., 2012; Richard et al., 2015; Richard et al., 2016). Results from these previous studies were used to build a spatial dataset of SFV infection presence and absence across much of the Congo Basin. These localities either represent the residence of tested individuals close to where they came in contact with non-human primates, such as in bushmeat markets and/or locations were they have hunted non-human primates in forests.

I used Biomod2 (Thuiller et al., 2013) to map and predict the occurrence of SFV infections in humans across the Congo Basin. Biomod2 represents a leap forward in distribution modeling techniques because it can be used to create robust ensemble models from up to 11 different distribution model types (Thuiller et al., 2013). Biomod2 allows users to generate distribution models from a presence/absence dataset and processed geospatial predicting factors using generalized linear model (GLM), generalized boosting model (GBM), generalized additive model (GAM), classification tree analysis (CTA), artificial neural network (ANN), surface range envelope (SRE or BIOCLIM), flexible discriminant analysis (FDA), multiple adaptive regression splines (MARS), random forests (RF), and Maxent (Phillips et al., 2006; Tsuruoka 2006). Each of these models uses different mathematical approaches to determine relationships between locations of presence and absence with underlying geospatial predicting layers. When each model is created, it can be projected across

a given study region using the same geospatial predicting factors to create a map that shows estimated probability of occurrence. Finally, an ensemble model can be created by averaging each model projection weighted by individual scores of their performance (receiver operating characteristic (Hanley et al., 1982), true skill statistic (Allouche et al., 2006), or Cohen's K (KAPPA: Monserud et al., 1992)), while also removing poorly-performing models based on a user-defined threshold. Using Biomod2 (Thuiller et al., 2013) and Biomod2EZ (Chapter 6)(Sesink Clee et al., In prep.), I created an ensemble model of human infections of SFV as a function of human presence and encroachment activities including population density, roads, active logging sites, agricultural plantations, urban expansion, and intensity of bushmeat hunting (Table 1) from 110 individual model projections in order to determine human activities that are most closely linked to SFV transmission from non-human primates to humans. Factors that contributed most to the models were then used to create a map layer showing human dimensions of zoonotic infection risk.

Modeling zoonotic diseases occurrence in non-human primates

Distribution models were created for wildlife infectious diseases in non-human primates across the Congo Basin using data from past studies that involved non-invasive sampling of primates by the collection of feces or post mortem samples from hunted carcasses and those found in the forest (Huijbregts et al., 2003; Leroy et al., 2004a; Van Heuverswyn et al., 2007a; Neel et al., 2010; Choudhary et al., 2013; Locatelli et al., 2016). I targeted studies with sampling after

year 2000 involving SIV (Santiago et al., 2002; Switzer et al., 2005; Van Heuverswyn et al., 2006; Neel et al., 2009; Rudicell et al., 2010; Locatelli et al., 2016), SFV (Hussain et al., 2003; Calattini et al., 2004; Calattini et al., 2007; Liu et al., 2008; Mouinga-Ondémé et al., 2012; Locatelli et al., 2016), and Ebola (Huijbregts et al., 2003; Walsh et al., 2003; Leroy et al., 2004a; Leroy et al., 2004b; Leroy et al., 2005; Pourrut et al., 2005; Bermejo et al., 2006; Lahm et al., 2007; Feldmann et al., 2011) in chimpanzees, gorillas, and mandrills (*Mandrillus sphinx*) in the Congo Basin that reported sampling localities with detected disease presence. Some studies involving sampling of wild primates with the aim of detecting disease/pathogen presence report a single coordinate for a sampling area or basecamp around which many samples were collected. Although this can simplify efforts in the field, these less detailed sampling coordinates can be difficult to synthesize for geospatial studies. For localities with multiple reported samples, absence was denoted only if all samples tested negative for viral presence.

Next, environmental predicting factors, including measures of climate, topography, and vegetation, were assembled from various sources to collectively describe the habitat (Table 2) (Long et al., 2001; Hijmans et al., 2005; Farr et al., 2007; Freedman et al., 2010; Dimiceli et al., 2011). These publically available layers were converted to a 30-arcsecond (about $1km^2$) resolution and transformed to the WGS84 projection and datum when necessary to ensure proper alignment with other layers used in later analyses. The assembled set of map layers were first tested for cross-correlation in order to remove redundant layers using pairwise Pearson correlation tests. Highly correlated ($R < -0.8$ or $R > 0.8$) layers were grouped into clusters and

the most descriptive layers in each cluster revealed from preliminary distribution models were kept for use in final distribution models.

Distribution models were created for SIV infecting the Central Chimpanzee (*P. t. troglodytes*), Eastern Chimpanzee (*P. t. schweinfurthii*), and the Western Lowland Gorilla (*G. g. gorilla*), SFV infecting chimpanzees (*P. t. troglodytes* and *P. t. schweinfurthii*), gorillas (*G. g. gorilla*), and mandrills (*Mandrillus sphinx*), and Ebola infecting chimpanzees (*P. t. troglodytes*) and gorillas (*G. g. gorilla*) using Biomod2 (Thuiller et al., 2013) and Biomod2EZ (Chapter 6)(Sesink Clee et al., In prep.). Each model by disease/host was created with 10 replicates for each individual model type for a total of 110 models that were then used to create an ensemble model with a performance threshold of 0 for true test statistic (TSS) (that ranges from -1 to +1) and 0.6 for receiver operating characteristic (ROC) (which ranges from 0.5 to 1). Individual presence models for each disease/host pair were rescaled to a range of 0 (absence) to 1 (highest probability of occurrence) and were used to create binary occurrence maps with conservative and speculative presence thresholds (0.75 and 0.5, respectively). Individual disease models were then combined for conservative and speculative estimates independent of host taxa to create maps of estimated zoonotic disease occurrence in non-human primate populations across the Congo Basin.

Risk of viral transmission from wild non-human primates

It was shown through my analysis of SFV infections in humans and several other studies of zoonotic transmissions, that bushmeat hunting along with

commercial logging increase human contact with wildlife, facilitating zoonotic disease crossover events from wildlife to humans (Bikié et al., 2000; Wolfe et al., 2005a; Laurance et al., 2006; Poulsen et al., 2009; Tieguhong et al., 2009). In order to assess their combined risk of zoonotic disease crossover, I sought to create a map layer that quantifies the additive levels of active logging and bushmeat hunting across the Congo Basin.

Recently, Hansen et al. (2013) utilized high-resolution satellite imagery to determine where forest loss has occurred around the world. One potential issue with incorporating this dataset into my study was the lack of distinction between logging versus tree loss from natural causes such as fire, which can be difficult to determine from raw satellite imagery. I assembled demarcations of logging concessions in Cameroon, Equatorial Guinea, Gabon, Central African Republic, Republic of the Congo, and Democratic Republic of the Congo (Mertens et al., 2016). Under the assumption that most forest loss within these concessions is caused by commercial logging, I used the boundaries of only logging concessions that have been active since 2000 as a mask to clip the map product of recent forest loss. The resulting map of forest loss within active logging concessions was then used to create a layer that represents the distance from active logging sites. Finally, a threshold was applied to highlight areas that are within 1 km from active logging sites to account for fringe effects of human presence in the forest such as logging worker camps.

Zeigler et al. (2016) recently used compiled data from studies reporting the off-take of mammals in the Congo Basin to calculate hunting intensity as a function of bushmeat off-take and diversity. Hunting data was collected from Cameroon, Gabon, Equatorial Guinea, Central African Republic, and Republic of the Congo and used to identify relationships with environmental and anthropogenic factors to predict where the highest levels of hunting occur. They found that regions of high hunting intensity were most correlated with proximity to roads, protected areas, and human population density. Their resulting map product shows bushmeat hunting pressure categorized as low, moderate, and high across the region. I used this layer by first omitting regions designated with low bushmeat hunting pressure in order to focus on areas where hunting was highest. Since the zoonotic diseases include in this study involve primarily forest-dwelling non-human primate hosts, I then clipped this hunting pressure map to include only locations with more than 30% tree density determined from MODIS satellite imagery (Dimiceli et al., 2011). Finally, I combined this model of bushmeat hunting intensity with close proximity to active logging sites across the Congo Basin to create a layer that highlights areas of highest potential for human-zoonoses contact. This layer was used in conjunction with individual disease models to identify key regions of highest disease crossover risk from non-human primates to humans across the Congo Basin.

Identifying areas of highest zoonotic crossover risk within a non-human primate zoonotic disease hotspot

Ensemble distribution models for Ebola, SIV, and SFV infecting chimpanzees, gorillas, and mandrills were combined to determine areas of highest overlap to create conservative and speculative primate disease hotspot maps. These layers were then compared using zonal statistics in ArcMap 10.1 (ESRI, 2013) with the new human dimensions of zoonotic disease transmissions layer to identify areas of highest crossover risk to humans where multiple diseases coincide with one another in non-human primates. Protected areas, if properly maintained and managed, may act as effective barriers between zoonotic disease in wildlife and humans, but most protected areas in Central Africa are open, lacking fences or manmade barriers at their borders, making entry difficult to control even when there are effective anti-poaching and encroachment patrols (Joppa et al., 2008). For the animals in these protected areas, open plans are important to avoid tampering with wildlife corridors and movement patterns (Bennett, 1999). Open-access protected areas are permeable, which can become an issue with nearby people because the resources inside are largely unmanaged and accessible when enforcement is lacking (Woodroffe et al., 1998). Finally, I compared protected area boundaries with maps showing combined primate disease occurrence that I produced using the estimated ranges of Ebola, SIV, and SFV in chimpanzees, gorillas, and mandrills in the Congo Basin.

Results

Anthropogenic activities linked to crossover of zoonotic disease from non-human primates to humans in the Congo Basin

Localities of people that were tested for SFV infections across the Congo Basin were used to create a model of zoonotic crossover from non-human primates as a function of different anthropogenic activities that may increase contact with wildlife (Appendix 2.1). The final ensemble model of SFV infections in humans was primarily driven by bushmeat hunting intensity (contributing 31.49% to the overall distribution), proximity to active logging sites (contributing 29.846%), and proximity to roads (contributing 22.31%). Human population density, urban extents, and proximity to agricultural plantations and croplands contributed very little to the ensemble model. Locations of SFV infections in humans across the Congo Basin appear to occur most often in areas where bushmeat hunting is high and distance to active logging sites and roads are relatively low (Appendix 2.1). Zeigler et al. (2016) reported that that proximity to roads was important in the creation of their bushmeat intensity layer, and a Pearson Correlation test revealed that bushmeat hunting intensity was positively correlated with distance from roads ($R = 0.72$, $p < 0.05$). High hunting pressure and close proximity to active logging sites were closely tied to SFV crossover, and used as indicators of increased risk of zoonotic crossover between non-human primates and humans in the Congo Basin.

Mapping human pressures that increase chances of zoonotic crossover from non-human primates

Our preliminary model of SFV infections in humans used to identify anthropogenic pressures indicative of zoonotic crossover from non-human primates, identified bushmeat hunting and commercial logging as the most important factors that predict locations where zoonotic crossover is most- and least- likely. By combining models of commercial logging with intensity of bushmeat hunting, I was able to create a map that identifies regions in the Congo Basin where human activities may increase the risk of zoonotic disease crossover from non-human primates (Figure 1). In the Congo Basin, Gabon and Republic of the Congo show considerably more overlap in logging and hunting activity than other countries. By contrast in Cameroon and Central African Republic, there is substantially more separation between these pressures, with little hunting occurring near large logging operations. Finally, most regions with the highest combined risk fall along the periphery of protected areas and close to major roads, while areas of lowest combined risk are primarily in remote, difficult to access regions (Appendix 2.2).

Modeling the distributions of zoonotic diseases in primates and assessing crossover risk to humans

Locality data from previous studies of wild chimpanzees, gorillas, and mandrills where samples were tested for the presence of Simian Immunodeficiency Viruses, Simian Foamy Viruses, or Ebola Viruses were collected into datasets of

presence and absence localities across the Congo Basin. These localities were used along with a set of environmental predictors including measures of temperature, precipitation, seasonality, vegetation, and topography (Table 2) (Long et al., 2001; Hijmans et al., 2005; Farr et al., 2007; Freedman et al., 2010; Dimiceli et al., 2011) to create distribution models using Biomod2 (Thuiller et al., 2013). Final models were projected across the Congo Basin, resulting in maps that provide estimates of disease occurrence at each grid cell (Appendix 2.3-2.11). Non-human primates infected with Ebola appear to occur throughout Gabon, extending into central Cameroon to the north (Figure 2, A and B). SFV infections in non-human primates are highest throughout almost all forested regions of Cameroon and Gabon, and also in parts of neighboring Republic of the Congo and Democratic Republic of the Congo (Figure 2, C and D). Finally, SIV infections were found to be highest across southern Cameroon and northern Gabon extending eastward along the Congo River into Democratic Republic of the Congo (Figure 2, E and F).

Distribution models from this study were combined, and areas of overlap were determined to create maps of overall disease occurrence across all study taxa using conservative and speculative estimates (0.75 and 0.5 probability thresholds, respectively). Combined disease presence maps were then compared to human dimensions of zoonotic disease using zonal statistics in ArcMap 10.1 (ESRI, 2013) to identify key regions of highest risk for zoonotic disease crossover from wild non-human primates to humans. Over 80% of this disease hotspot coincides with areas of high to critical levels of human pressures associated with zoonotic disease crossover risk from non-human primates. The average threat across this hotspot is

moderate to high (Figure 3), with a clear gradient of increasing threat to humans leading to a core area of highest risk in northeastern Gabon and neighboring portions of Cameroon and Republic of the Congo, 19.2% of which falls within protected areas (Figure 3). Mwagne National Park and Ivindo National Park in northeast Gabon and Lossi Gorilla Sanctuary and the Odzala-Kokoua National Park in neighboring northwest Republic of the Congo all fall within a critical zone that marks the coincidence of high levels of bushmeat hunting and commercial logging with high probability of occurrence of zoonoses in non-human primates. Lopé National Park and Waka National Park in Gabon and the Dja Faunal Reserve in southern Cameroon appear to overlap with mid-high levels of human pressures along with zoonotic presence in primates.

Discussion

Anthropogenic drivers of zoonotic crossover from non-human primates

Over two-thirds of infectious diseases in human have origins in wildlife populations (Taylor et al., 2001; Calvignac-Spencer et al., 2012; Wang et al., 2014), and it is especially important to understand how these diseases crossover to people in rural areas where contact with wildlife can be frequent. The increased contact with wildlife due to proximity to forests that sustain high levels of biodiversity and human population expansion across Central Africa (Starkey, 2004; Wolfe et al., 2005a) makes the Congo Basin a key region to study the effects of different human activities on disease crossover risk. This study used Simian Foamy Virus infections in humans contracted from wild non-human primates to create a predictive model

as a function of anthropogenic pressures. This model showed that sites of SFV infections in the Congo Basin occur primarily in areas of high bushmeat hunting, close proximity to active logging sites, and close proximity to roads. These areas are often far from major urban centers where bushmeat hunting is a main source of income and sustenance for local people.

The largely intact, biodiversity-rich forests of Central Africa, along with the recent willingness of governments to lease land for commercial logging, have drawn in large operations to harvest trees for lumber (Laporte et al., 2007). In this region, selective logging is much more common than clear-cutting practices within logging concessions due to the difficulty in reaching remote areas, and the cost of transportation (Laporte et al., 2007). Clear-cutting, although devastating to the environment, can limit the exposure of humans to wildlife infectious diseases, while selective logging is a much more intimate process, involving transporting a group of workers out to remote regions of the forest where they typically select the best trees and harvest them on foot (Patz et al., 2004; Wolfe et al., 2005a). The recent expansion of logging efforts across Central Africa brings people increasingly closer to wildlife, with recent analyses showing that in Cameroon more than half of previously remote forests are now within 10km from an active logging road (World Resources Institute, 2016b). Of all African countries, Gabon and Republic of the Congo rank first and second for the most forested countries on the continent (World Resources Institute, 2016b), but only 11% of forests in Gabon fall within protected areas, while much of the remaining forest has been leased as concessions reserved for some form of logging (Laporte et al., 2007; World Resources Institute, 2016b).

This mosaic sorting of land use leaves Gabon with isolated pockets of protected areas that are surrounded by active logging sites (Rayden et al., 2010). Similar patterns, with protected areas surrounded by logging concessions, can be seen in southern Cameroon, Republic of the Congo, and Democratic Republic of the Congo. This increased activity in what were formerly remote forests, also drastically increases the ease of access for bushmeat hunters to untapped sources of wildlife (Barnes, 2002).

Bushmeat is a major part of the diet of people throughout tropical forested areas today, particularly in Central Africa where local people each consume about 250 grams per day and the total off-take from the forests is estimated to be over 4.5 million tons per year (Fa et al., 2002; Wolfe et al., 2005a; Koerner et al., 2016). Unfortunately, bushmeat hunting has transitioned from a source of sustenance for people in rural areas to include commercial hunting due to high demand from bushmeat consumers in large cities, with an estimated 90% of off-take from forests destined for urban bushmeat markets (De Merode et al., 2006). This shift is partly due to the increased availability of firearms, ease of access to forests by newly built roads, increased demand for bushmeat from urban centers where it is treated as a delicacy for the wealthy, and lack of enforcement, hunting bans, and other restrictions in areas where it is illegal to hunt (Milner-Gulland et al., 2003; Warchol, 2004; Abernethy et al., 2013; Robinson et al., 2013; Cronin et al., 2015). This combination of increased demand and ease of access to forests, results in humans coming into contact with wildlife more frequently than ever before, including those infected with zoonoses (Daszak et al., 2001; Wolfe et al., 2005a; Karesh et al., 2009).

The chain of people from the hunters in the forests to porters, middle-men, bushmeat market workers, and consumers can all come in contact with a single carcass, allowing pathogens to spread to multiple individuals over a relatively large distance in a short amount of time (Keusch et al., 2009).

The Congo Basin is a hotspot of global importance for biodiversity, especially along the Atlantic coastal forests of Cameroon, Equatorial Guinea, and Gabon extending eastward to Democratic Republic of the Congo, housing more than 200 species of mammals including nearly 30 species of non-human primates (Oates et al., 2004; Jenkins et al., 2013a; Jenkins et al., 2013b). Logging operations in this region appear to have promoted bushmeat hunting since the boom of commercial logging in the early 1980s (Robinson et al., 1999; Walsh et al., 2003; Roy et al., 2005; Zhang et al., 2005). Today, logging is expected to increase throughout the Congo Basin with the completion of the Kribi Deep Sea Port in southwest Cameroon near the border with Equatorial Guinea and Gabon that will be serviced by a railway to facilitate transport of natural resources from the continental interior to the coast in a supply chain reaching global markets (Tabi et al., 2011; Schwartz et al., 2012; Fisken, 2013). Large-scale logging operations that thrive in Cameroon, Republic of the Congo, Gabon, and Democratic Republic of the Congo increase forest access from the creation of new roads and trails used for the transportation of lumber. When large logging operations became commonplace in this region, hunters were given an entrance route to access forests that were previously out of reach (Laurance et al., 2006). The roads built for lumber transportation routes are often used by hunters that hitch rides with logging workers so that they can hunt for valuable mammals

deep in the forests, and then hitch rides back out with carcasses to sell at bushmeat markets. Some hunters also settle along active logging transportation routes and simply sell their spoils from the forests to passing logging company staff and others traveling in the area (Robinson et al., 2013). Thus, the close association of active logging efforts and increased hunting for bushmeat makes for a critical combination that increases the risk of zoonotic disease crossover from wildlife to humans.

Identifying key regions of highest zoonotic crossover risk from non-human primates for Ebola, SFV, and SIV

Using recent research on the state of bushmeat hunting and commercial logging in the Congo Basin, I was able to create a map of combined threats to identify key regions where increased exposure of humans to wildlife increase the risk of zoonotic disease transmission. The most critical regions for zoonotic transmission risk occur across southern Cameroon into Gabon and eastward along the Congo River between Republic of the Congo and Democratic Republic of the Congo (Figure 3). This map of compounded human activities that were shown to be associated with disease crossover from non-human primates was used to determine where humans are most at risk to come in contact with Ebola, SFV, and SIV today.

Distribution models for all three diseases included in this study show some of the highest estimated presence (and overlap with disease transmission risk) at the border between Gabon, Republic of the Congo, and Cameroon. SFV was modeled in chimpanzees, gorillas, and mandrills, and the core of combined presence appears to have the largest coverage, encompassing much of central/southern Cameroon,

Equatorial Guinea, and Gabon (Figure 2, C and D). This large distribution of occurrence is not surprising since SFVs are found in essentially all non-human primates across Africa (Murray et al., 2006). With its large distribution and presence in many primate species, it is one of the most common zoonoses to transfer between non-human primates and hunters in tropical Africa (Heneine et al., 1998; Switzer et al., 2004; Wolfe et al., 2004a; Jones-Engel et al., 2005; Calattini et al., 2007). Previous studies have shown that infected primates have high concentrations of SFVs in their saliva (Falcone et al., 1999; Linial, 1999), and in a study based in Cameroon, more than 35% of hunters that were bitten by wild chimpanzees or gorillas, tested positive for the virus (Calattini et al., 2007). In an infected human, SFVs start by acting similarly to how they do in non-human primates. The viruses replicate in the same fashion as other retroviruses, and then target specific cells and tissues to form multi-nucleated syncytia (Linial, 1999). In non-human primates, there is evidence that SFV infections may increase susceptibility to additional infections (Choudhary et al., 2013). Many zoonotic diseases that can infect humans do not transfer between humans, and instead establish themselves in populations by repeated transmissions from wildlife in a pattern known as "viral chatter" (Wolfe et al., 2004b). This pattern is commonly seen where pathogens ping repeatedly into human populations causing outbreaks without horizontal transmission, and is how SFV has infected people across parts of rural Central Africa (Wolfe et al., 2005a; Switzer et al., 2011). For a disease like this to persist in human populations, they must be infected repeatedly from contact with

infected wildlife. Increased bushmeat hunting across the region results in more viral chatter, and more infected humans over time (Keusch et al., 2009).

Of the three diseases included in this study, Ebola is the most virulent with recent outbreaks in both wildlife and humans (Muyembe-Tamfum et al., 2012; Fauci, 2014). Outbreaks of Ebola in humans have appeared in West and Central Africa since the 1970s, but the most recent outbreaks from around 2012 through 2016 have been the most intense, with more than 28,000 infections causing over 10,000 deaths (Leroy et al., 2009; Muyembe-Tamfum et al., 2012; Briand et al., 2014). Through these outbreaks, new research suggests that frugivorous bats may act as a natural reservoir for the virus, and transmission is likely to occur from consuming partially eaten fruits (Leroy et al., 2005; Leroy et al., 2009). This same transmission route may explain the transfer of the virus from bats to non-human primates such as chimpanzees, gorillas, and mandrills. The distribution models for Ebola virus in non-human primates accurately highlight regions where there have been recent outbreaks of the virus causing declines in local populations across northeastern Gabon and western Republic of the Congo (Figure 2, A and B) (Walsh et al., 2003; Leroy et al., 2005; Bermejo et al., 2006). Additionally, the speculative model (Figure 2B) from this study overlaps entirely with a recent Ebola distribution model in humans in this region (Pigott et al., 2014), suggesting the effectiveness of the natural reservoirs and animal hosts for this disease in aiding spread to multiple other host taxa. Understandably, the model I created for Ebola crossover risk from non-human primates does not encompass the entirety of the Ebola model for humans in general, due to transmission of the virus between humans and from other sources, but they

both share comparable Ebola risk estimates where overlap does occur. It is important to consider that if the outbreaks in chimpanzees and gorillas occurred from transmission via infected frugivorous bats in close proximity to them, humans in the same region could also be at threat of infection both from contact with infected non-human primates and by transmission from frugivorous bats.

Simian Immunodeficiency Viruses are thought to have crossed over into humans originally in the 1920s near Kinshasa, Democratic Republic of the Congo (then Belgian Congo) most likely via blood-to-blood contact while hunters were butchering chimpanzee carcasses. By combining distribution models for SIVs in chimpanzees and gorillas across Central Africa, I was able to create risk maps that include both strains of SIV that are known to have crossed the species boundary into humans that lead to HIV-1 types M, N, and O in the past (Gao et al., 1999; Keele et al., 2006; Van Heuverswyn et al., 2007b; Locatelli et al., 2014; D'arc et al., 2015). SIVs are estimated to be present in non-human primates across southern Cameroon and northern Gabon stretching eastward through Republic of the Congo into Democratic Republic of the Congo. Throughout this estimated range, pockets of high crossover risk to humans are common except in northeastern Republic of the Congo where it appears that there are low levels of both hunting and commercial logging.

Additionally, the bushmeat hunting intensity estimate by Zeigler et al. (2016) revealed an interesting pattern where the highest levels of hunting occur in rings around the periphery of protected areas, with moderate hunting within their borders (Figure 1). They explained that the high levels of hunting within protected

areas might be due to close proximity to major roads that can provide easier access for illegal hunting within them. It has been shown that human settlements tend to congregate around the boundaries of protected areas, especially in Central Africa (Wittemyer et al., 2008), which may be driven by the availability for jobs in enforcement, Integrated Conservation and Development Projects (ICDPs) (Alpert, 1996; Oates, 1999), or possibly because of frequent road traffic for the sale of various goods including bushmeat (Willcox et al., 2007). The pattern of high hunting intensity around protected areas may be due to these rapidly growing populations being deterred from hunting excessively within these protected areas, and the intensification of hunting effort to surrounding "unprotected" areas. This could be a considerable issue for conservation planning because protected areas in Central Africa are typically open, allowing animals to move freely across their borders. Although, if properly managed, this situation may be able to provide local people in rural parts of the Congo Basin with the protein they require, if protected areas act as sources for wildlife that spill past their borders where they could be hunted (Wilkie et al., 1999).

Potential approaches to reduce zoonotic crossover risk in the future

Protected areas in the non-human primate zoonotic hotspot identified in this study near the shared boundaries of Cameroon, Gabon, and Republic of the Congo, may play an important role in preventing future disease crossover events. Local people that live along the periphery of protected areas in the developing countries of Central Africa are often among the poorest in the region (Laboy-Nieves et al.,

2008) and most of them rely on the forests around them for sustenance (Starkey, 2004). Within protected areas across this region, it is common to find active hunting, selective logging, and grazing by domesticated cattle (Bruner et al., 2001; Brashares et al., 2004). It appears that protected areas that fall within the critical zone of disease transmission risk from non-human primates in this study have moderate to high levels of human presence via a combination of bushmeat hunting and logging within their borders (Figure 3, C). Increased enforcement of protected areas such as Mwagne National Park, Odzala-Kokoua National Park, and Ivindo National Park in Gabon and Republic of the Congo and efforts to reduce demand for bushmeat may play large roles in limiting human exposure to zoonoses from non-human primates. Increased enforcement of illegal activities within protected areas also serves the dual purpose of increasing protection of the animals under threat from excessive hunting that these areas were established to conserve. Bushmeat hunting is a complicated issue since people in rural parts of Central Africa often lack access to other sources of protein, and thus, rely on resources in forests for sustenance. While sustainable hunting of certain species appears to be possible in some situations (Waite, 2007; Cronin et al., 2015), it would require substantial shifts in preference from the demand of urban centers to be successful at large scales (Brashares et al., 2011). Decreasing demand for bushmeat in urban settings by education of its risks and increased supply of alternative sources of protein may reduce hunting in forests to feed people in rural areas, and reduce the risk of zoonotic disease spread while simultaneously protecting wildlife (Wilkie et al., 1999; Wolfe et al., 2005a).

Figures

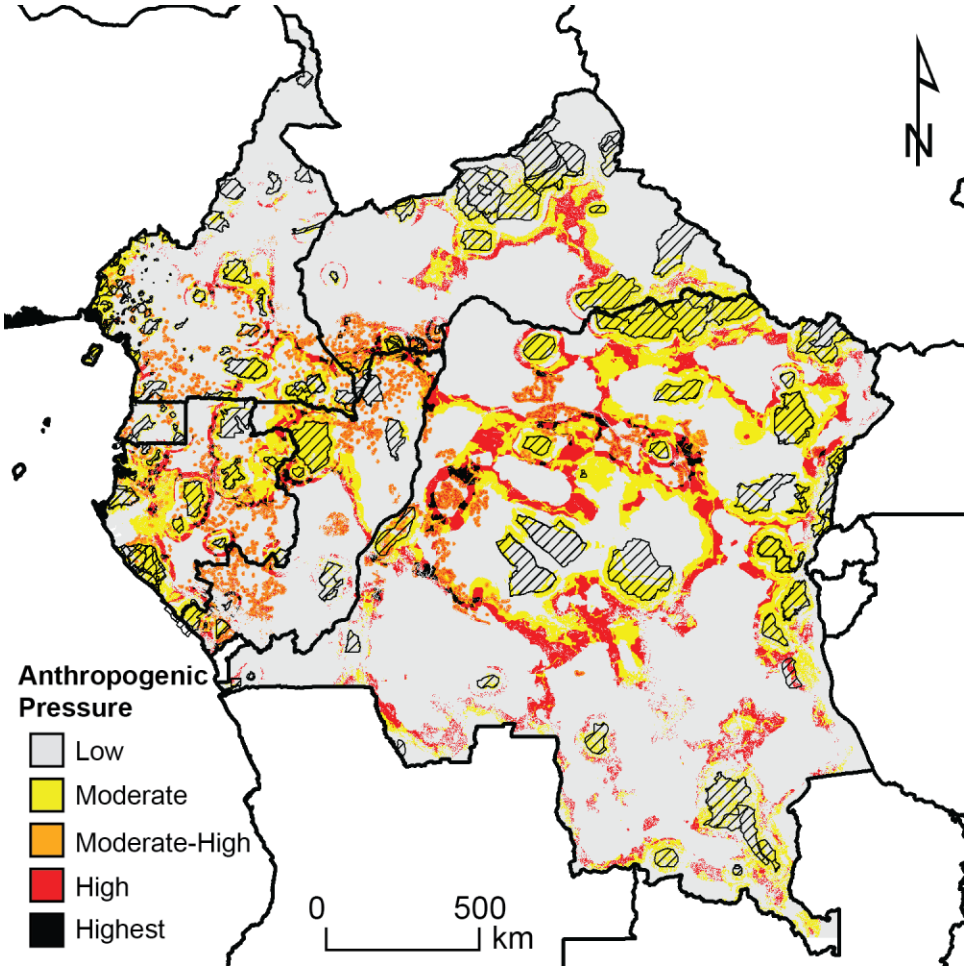

Figure 1. Map of anthropogenic pressure that includes logging and bushmeat hunting in forested regions, which brings humans in close contact with wildlife that can harbor zoonotic diseases and pathogens. Hatched areas represent protected areas.

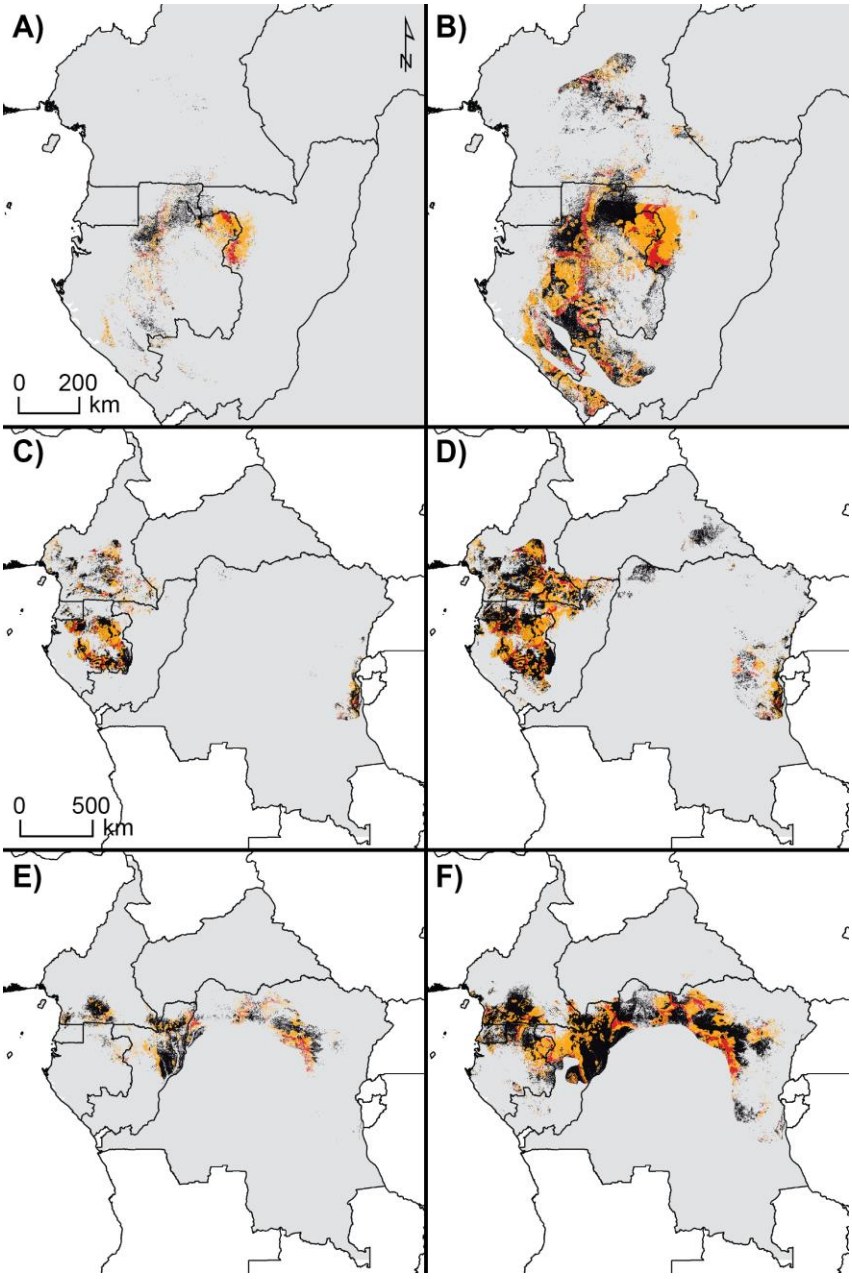

Figure 2. Distribution models for Ebola (A and B), Simian Foamy Virus (C and D), and Simian Immunodeficiency Virus (E and F) where the first column maps are conservative, showing only areas with more than 75% probability of presence, and second column of maps are speculative, showing only areas with more than 50% probability of presence. Gray indicates disease absence, Black indicates disease presence only, while Orange and Red indicate disease occurrence with moderate and high levels of hunting/logging.

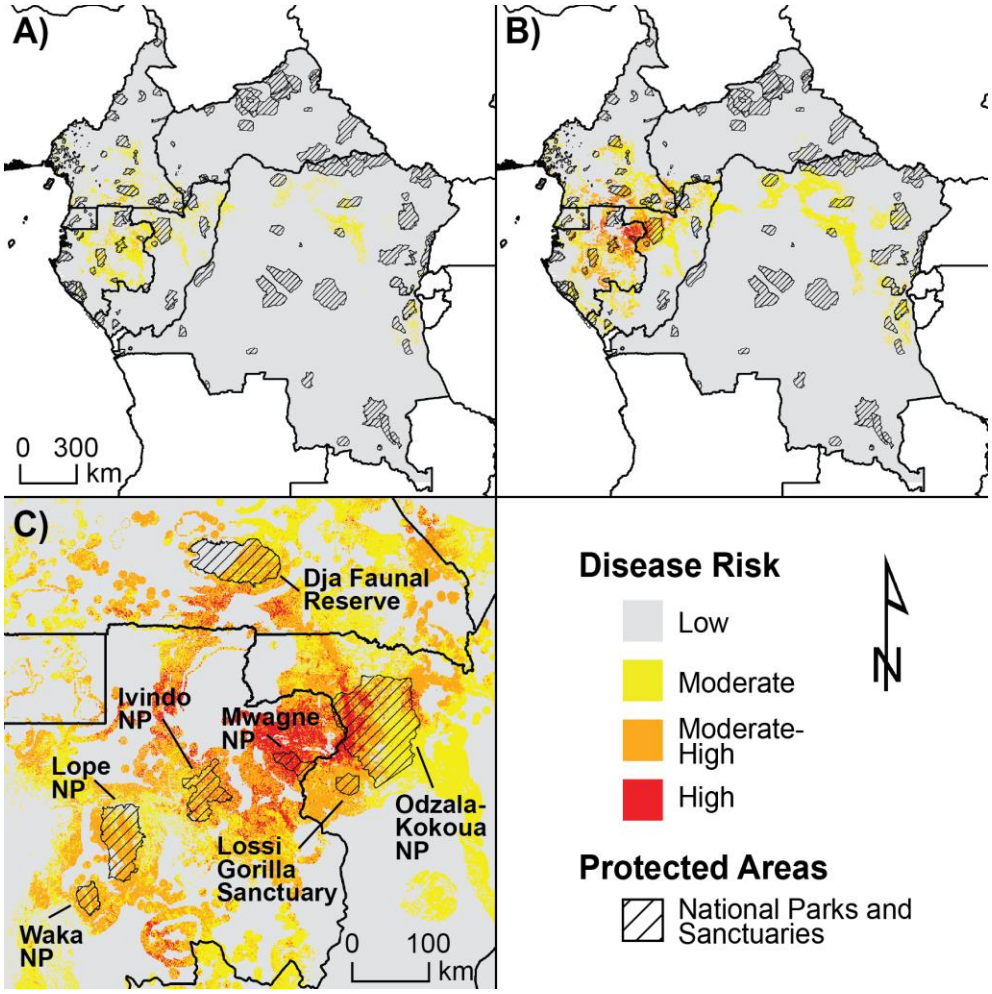

Figure 3. Conservative (A) and speculative (B and C) zoonotic crossover risk maps for combined presence of Ebola, Simian Foamy Virus, and Simian Immunodeficiency Virus in primates in relation to protected areas (national parks and sanctuaries).

Tables

Table 1. Measures of anthropogenic pressures used to model the relationship with human cases of Simian Foamy Virus in the Congo Basin

Variable Name	**Source**
Human Population Density	(Ciesin, 2008; Landscan™, 2010; Ciesin, 2014)
Road Networks	(Ciesin, 2013)
Agricultural Plantations and Croplands	(World Resources Institute, 2016a)
Urban Extents	(Ciesin, 2014)
Active Logging Sites	(Modified from Hansen et al., 2013)
Bushmeat Hunting Activity	(Ziegler et al., 2016)

Table 2. Environmental predicting factors used to create distribution models for zoonosis in non-human primate populations.

Variable Type	Variable Name	Source
Climatic Factors	Bio1 – Annual Mean Temperature	WorldClim; (Hijmans et al., 2005)
	Bio2 – Mean Diurnal Range	
	Bio3 – Isothermality	
	Bio4 – Temperature Seasonality	
	Bio5 – Max. Temp. of the Warmest Month	
	Bio 6 – Min. Temp. of the Coldest Month	
	Bio 7 – Temperature Annual Range	
	Bio8 – Mean Temp. of the Wettest Quarter	
	Bio9 – Mean Temp. of the Driest Quarter	
	Bio10 – Mean Temp. of the Warmest Quarter	
	Bio11 – Mean Temp. of the Coldest Quarter	
	Bio12 – Annual Precipitation	
	Bio13 – Precipitation of the Wettest Month	
	Bio 14 – Precipitation of the Driest Month	
	Bio15 – Precipitation Seasonality	
	Bio 16 – Precipitation of the Wettest Quarter	
	Bio 17 – Precipitation of the Driest Quarter	
	Bio18 – Precipitation of the Warmest Quarter	
	Bio19 – Precipitation of the Coldest Quarter	
Topographic Factors	Elevation	NASA SRTM; (Farr et al., 2007)
	Slope	Derived from above In ArcMap 10 (ESRI, 2013)
Vegetation Indices	Percent Tree Cover	MODIS; (Dimiceli et al., 2011)
	NDMAX – Max. Annual NDVI (Normalized Difference Vegetation index)	MODIS; (Freedman et al., 2010)
	NDMEAN – Mean Annual NDVI	
	NDGR – Max. NDVI of Greening Season	
	NDBR – Max. NDVI of Least Green Season	
	NDGRBR – NDVI Seasonality	
	QMEAN – QSCAT (Surface Moisture Content) Annual Mean	Quick Scatterometer; (Long et al., 2001)
	QSTD – QSCAT Standard Deviation	

Chapter 3: Niche divergence between Simian Immunodeficiency Virus (SIV) and Simian Foamy Virus (SFV) in chimpanzees across Equatorial Africa that persists under climate change

Abstract

It has become increasingly important for studies of wildlife infectious diseases to better understand how environmental factors may influence their occurrence and shape their distributions across the landscape. Wildlife infectious diseases, especially those that transmit via direct contact between individuals, are constrained to the ecological niche of their animal hosts. Simian Immunodeficiency Viruses (SIVs) infect both Central Chimpanzees (*Pan troglodytes troglodytes*) and Eastern Chimpanzees (*P. t. schweinfurthii*) in central Africa, but for reasons that are still unclear, they appear to have an uneven distribution across the region and apparent absence in the Nigeria-Cameroon Chimpanzee (*P. t. ellioti*) even with ongoing migration with *P. t. troglodytes*. Recent evidence suggests that SIVs infecting chimpanzees (SIV*cpz*) in Cameroon may occupy an ecological niche that is different from that of the closely related widespread Simian Foamy Virus (SFV*cpz*), but it was not known whether this pattern is localized to this region or if it is a more general pattern.

In this study, I assembled SIV*cpz* and SFV*cpz* occurrence data from previously published studies of wild chimpanzees, expanding coverage across the full ranges of *P. t. ellioti*, *P. t. troglodytes,* and *P. t. schweinfurthii* to better understand the spread of these pathogens across Central Africa. Using robust ensemble modeling techniques,

I predicted the occurrence of SIV*cpz* and SFV*cpz* across this region to determine if niche divergence persists in chimpanzees outside of Cameroon. Ensemble occurrence models were created under both present climatic conditions and future climate models from the TERRA6-AR5 multi-model ensemble. Niche comparison and correlation tests showed that the environmental space that SIV*cpz* and SFV*cpz* occupy are largely divergent and non-overlapping throughout the ranges of their chimpanzee hosts across central Africa, similar to the pattern observed in Cameroon. To best summarize future models of each virus, I created habitat suitability change maps that identify regions of increasing and decreasing suitability over time. Final habitat suitability change maps show an inverse relationship between SIV*cpz* and SFV*cpz* occurrence, with most regions of increasing suitability for one virus showing a corresponding decrease in suitability for the other virus. The causes of geographic separation between these two pathogens infecting chimpanzee hosts are unclear, but this study suggests that ecological and environmental factors are important in determining their distributions. Other factors that may also be important in defining this separation include viral competition, geographic boundaries limiting host taxa dispersal, lethal coinfection, viral competition, and localized immunity.

Introduction

Simian Immunodeficiency Viruses (SIVs) were first isolated in 1985 from captive rhesus macaques (*Macaca mulatta*) that were exhibiting AIDS-like symptoms (Daniel et al., 1985). Since its original discovery, SIVs have been found to

infect 45 species of non-human primates, including chimpanzees of Central Africa (Peeters et al., 2002; Locatelli et al., 2014). SIV naturally occurs in both the Central Chimpanzee, *Pan troglodytes troglodytes* (SIVcpz*Ptt*), and the Eastern Chimpanzee, *P. t. schweinfurthii* (SIVcpz*Pts*), but appears to be absent in the other chimpanzee subspecies: the Nigeria-Cameroon Chimpanzee, *P. t. ellioti*, and the Western Chimpanzee, *P. t. verus* (Figure 1) (Sharp et al., 2005; Locatelli et al., 2014). Chimpanzee SIV (SIV*cpz*) is a recombinant virus that appears to be derived from SIV*gsn* from Greater Spot-Nosed Monkeys (*Cercopithecus nictitans*) and SIV*rcn* from Red-Capped Mangabeys (*Cercocebus torquatus*), since SIV*cpz* contains non-overlapping regions of its genome most closely related to these other strains (Sharp et al., 2001). Crossover and recombination may have occurred from contact while chimpanzees were hunting these monkey taxa (Bailes et al., 2003). SIVcpz*Ptt* (infecting *P. t. troglodytes*) subsequently crossed over to humans in southern Cameroon most likely via contact through bushmeat hunting, where it developed into HIV-1 groups M and N (Santiago et al., 2002; Keele et al., 2006) and, through a separate transmission from Western Lowland Gorillas developing into SIV*gor* (Van Heuverswyn et al., 2006; D'arc et al., 2015). SIVs are transmitted by contact with bodily fluids such as blood, saliva, urine, and semen from infected individuals and it appears that it may transmit horizontally between adults and, rarely, vertically from mother to child via milk (Apetrei et al., 2004). Although early studies reported the lack of adverse symptoms in chimpanzees infected with SIVs, infected wild individuals have since been found developing AIDS-like symptoms that can lead to death (Novembre et al., 1997; Keele et al., 2009; Etienne et al., 2011). In infected

non-human primates, SIVs infect CD4+ T cells that play a large role in immune response (Keele et al., 2009). Infected cells undergo apoptosis, leading to progressive deterioration of immune system response by loss of CD4+ T cells over time, resulting in an immunodeficiency syndrome (Novembre et al., 1997).

Simian Foamy Viruses (SFVs) are another retrovirus closely related to SIV that occurs as species-specific variations found in all African non-human primates including monkeys and apes (Meiering et al., 2001; Hussain et al., 2003). Studies investigating the origins of SFVs infecting non-human primates revealed very similar phylogenies and substitution rates between SFVs and hosts genomes, suggesting that SFVs have undergone co-speciation with their primate hosts for more than 30 million years (Verschoor et al., 2004; Switzer et al., 2011). It has been proposed that SFV is transmitted via saliva during aggressive behavior between primates (Mouinga-Ondémé et al., 2012; Stenbak et al., 2014). Like other retroviruses, SFVs integrate their genetic material into the host genome and rely on host cell factors for viral gene expression (Linial, 1999). In infected individuals, SFVs cause the fusion of cells into multi-nucleated syncytia that have a 'foamy' appearance, but do not appear to be directly harmful to their primate hosts (Linial, 2000; Meiering et al., 2001; Murray et al., 2006). Although it is not known to directly cause adverse symptoms in humans, its high prevalence both in captive and wild individuals (Meiering et al., 2001; Murray et al., 2006; Stenbak et al., 2014) aids its ongoing transmission to human populations, making it a concern for human public health since its affects are not fully understood (Liu et al., 2008; Betsem et al., 2011; Huang et al., 2012; Mouinga-Ondémé et al., 2012).

Locatelli et al. (2016) completed a comprehensive study of SIV*cpz* in Cameroon, including samples from the putative SIV+/- boundary at the Sanaga River to better understand the apparent absence of SIV in *P. t. ellioti*, and to examine the factors that contribute to its uneven distribution in this region. One of the most intriguing findings of this study was that there appears to be an inverse relationship between the geographic distributions of SIV and SFV in chimpanzees of this region, even within neighboring Central Chimpanzees (*P. t. troglodytes*): areas exhibiting a high prevalence of SFV infections had few or no SIV infections. Additionally, SFV occurrence was predicted across the majority of *P. t. ellioti* range, where there is no evidence of SIV occurrence (Locatelli et al., 2014). Similarly, within the range of *P. t. troglodytes* in southern Cameroon, prevalence and occurrence models showed very little overlap between SFV*cpz* and SIV*cpz*. There are several potential causes that may have led to the geographic separation of these viruses in Cameroon including localized resistance or immunity, some level of viral competition within hosts where the presence of one makes a host insusceptible to the other virus, long-standing geographic separation of infected populations of chimpanzees that limit the chance of contact and transmission, or co-infection may be lethal or increase the virulence of one virus leading to mortality (Locatelli et al., 2014).

Predicting the occurrence of wildlife infectious diseases across the landscape is central to understanding relationships with the environment, geographic range, and potential risks to host taxa, but it is important to also consider how these relationships and distributions may shift over time under threat of climate change (Wiens et al., 2009; Gallana et al., 2013). Since SIV*cpz* and SFV*cpz* are not free-living,

distributions of these infections under climate change rely on host taxa niche requirements. As the climate changes overtime, host species can either shift their ranges to track their optimal climatic niche, persist in original locations if they are plastic enough to withstand environmental change, or risk extinction due to their inability to shift ranges (Thomas et al., 2004; Harvell et al., 2009; Cahill et al., 2012). All of these possible responses to climate change affect the movement of infectious diseases that these host species carry. Over time, areas of disease absence may turn into a hotspot of presence, and overall trends may change resulting in disproportional disease range estimates and overall disease spread (Jones et al., 2008).

The potential inverse geographic pattern between SIV*cpz* and SFV*cpz* with little overlap identified by Locatelli et al. (2016) only included *P. t. ellioti* and a small portion of the *P. t. troglodytes* range. It remains unclear whether this pattern holds true in other chimpanzee populations (i.e. throughout the entire range of *P. t. troglodytes* and *P. t. schweinfurthii*). Thus, this study aimed to: (1) determine whether niche separation between SIV*cpz* and SFV*cpz* occurrence is a broad-scale pattern that encompasses some or all of the ranges of *P. t. ellioti*, *P. t. troglodytes*, and *P. t. schweinfurthii*, (2) identify if and how environmental factors contribute to the distribution of each pathogen across the study area, and (3) address how climate change might alter the distribution of these viruses and their geographic relationship with one another in the future, potentially maintaining their differential spread throughout chimpanzee populations.

Methods

Disease occurrence and environmental predicting layers collection and processing

Localities of SIV and SFV presence/absence in chimpanzees of Cameroon and Nigeria (*P. t. ellioti* and *P. t. troglodytes*) (Locatelli et al., 2016) were expanded to include samples from previous collections that together encompass the full ranges of *P. t. troglodytes* and *P. t. schweinfurthii* using data from previous field studies with sampling performed after year 2000 (Santiago et al., 2002; Sharp et al., 2005; Calattini et al., 2006; Keele et al., 2006; Van Heuverswyn et al., 2007a; Liu et al., 2008; Keele et al., 2009; Neel et al., 2010; Mouinga-Ondémé et al., 2011; Li et al., 2012; Souquiere et al., 2012; Boué et al., 2015). Sites where the virus is present were categorized as 'pathogen presence' localities, while sites where all samples tested negative for viral presence were categorized as 'pathogen absence' localities. For publications that did not provide detailed coordinates for sample locations in supplemental figures or from communication with corresponding authors, points were extracted from georeferenced maps using ArcMap 10.1 (ESRI, 2013).

Since most wild populations of chimpanzees are not habituated, non-invasive sampling was performed by testing collecting feces for viral presence by identifying virus-specific antibodies using INNO-LIA I/II score confirmation tests (Locatelli et al., 2016) and enhanced chemiluminescence Western blotting approach with reverse transcriptase PCR (Sharp et al., 2005; Keele et al., 2006). These methods have been used successfully with non-invasive sampling to determine whether individuals are infected with various pathogens (Neel et al., 2010). While there are some local studies on *P. t. verus*, mostly in Taï National Park in Côte d'Ivoire,

localities lacked the geographic coverage to accurately capture pathogen presence across its entire range, and was omitted from this study. Small-scale studies of *P. t. verus* populations have identified the presence of SFV*cpz* (Morozov et al., 2009), but there is no evidence for the presence of SIV*cpz* (Prince et al., 2002; Leendertz et al., 2011). SIV*cpz* absence in *P. t. verus* may be explained by *P. t. troglodytes* acquiring it after splitting from *P. t. ellioti* and *P. t. verus* about 235-470kya years ago (Fünfstück et al., 2015) along with lack of gene flow between *P. t. verus* and other subspecies of chimpanzees (Groves, 2001; Oates et al., 2008; Gonder et al., 2011; Bowden et al., 2012; Prado-Martinez et al., 2013; Locatelli et al., 2014; Locatelli et al., 2016).

In order to create ecological niche models of SIV and SFV occurrence in chimpanzees under future climatic conditions, I first modeled their distributions in chimpanzees under present conditions using bioclimatic measures that include temperature, precipitation, and seasonality (Hijmans et al., 2005). The initial set of 19 bioclimatic layers was trimmed after identifying clusters of significantly correlated layers using pairwise Pearson correlation tests implemented in ENMtools (Warren et al., 2010). Layers were clipped to the distributional range of host taxa (*P. t. ellioti*, *P. t. troglodytes*, and *P. t. schweinfurthii*) (Humle et al., 2016) with an additional buffer of 50 kilometers in order to capture fringe habitats and allow for deviations from estimated ranges.

Ecological niche modeling procedure

Niche models for each subspecies specific virus were created using Biomod2 (Thuiller et al., 2013) in conjunction with Biomod2EZ (Chapter 6)(Sesink Clee et al.,

In prep.) for additional visualizations. Biomod2 is a package implemented in the R statistical framework (R Development Core Team, 2016) that can be used to create up to 11 different niche models using the same set of presence/absence points and map layers of environmental predicting factors. Each model can be tested for its accuracy and predictive power using: area under the relative operating characteristic curve (ROC)(Hanley et al., 1982), Cohen's K (KAPPA: Monserud et al., 1992), and the true skill statistic (TSS)(Allouche et al., 2006). From this information, one can select the model with the best "fit" or, alternatively, use model accuracy values as a weighting metric to create an ensemble model (Thuiller et al., 2009; Thuiller et al., 2013). Distribution models were created using 10 replicates for each of the 11 individual model methods resulting in 110 potential models that could be incorporated into final ensembles. Poorly performing models with TSS (that ranges from -1 to +1) less than 0 or ROC (which ranges from 0.5 to 1) less than 0.6 were excluded from ensembles, and the remaining models were combined using a weighting scheme using both ROC and TSS scores.

Niche model divergence testing

Distributions of SIV*cpz* and SFV*cpz* occurrence within each of the three chimpanzee subspecies were compared using pairwise niche divergence tests with an approach adopted from ENMtools (Warren et al., 2010) implemented using Biomod2. For each replicate, locality data for each virus to be compared were combined and then randomly resorted into two new pseudo-populations of the same sizes using R (R Development Core Team, 2016). Biomod2 (Thuiller et al.,

2013) was used to create an ensemble model projection for each of the new pseudo-populations, which were then tested for overlap using Schoener's *D* (Warren et al., 2010) and the *I* test statistic (Warren et al., 2008). A null distribution of *D* and *I* overlap values were created from the results of 50 pseudo-population niche comparisons. Significant deviations of observed values of *D* and *I* from comparisons of the true populations from the respective null distribution indicate that the niches occupied by the two populations under consideration are significantly divergent (Warren et al., 2010). If the observed values of *D* and *I* from the comparison of true population models fall within the range of the null distribution, the two populations are said to occupy significantly similar niches. Observed overlap values were compared to their respective null distributions using a student t-test in R (R Development Core Team, 2016). Due to the computational increase from using a single modeling technique (eg. Maxent by Sesink Clee et al., 2015) to Biomod2 ensemble modeling, 50 replicates were used instead of 100.

Assessing changes in habitat suitability

Biomod2 was used to project present niche models under four different climate change RCPs (2.6, 4.5, 6.0. and 8.5) each for years 2030, 2050, and 2080 using the same type of bioclimatic layers used to create present models. Future climate models based on the International Panel on Climate Change (IPCC) fifth assessment (AR5) were obtained from the TERRA6-AR5 climate model aggregate (Chapter 5)(Sesink Clee, In prep.): a multi-model ensemble of 20 different global

climate models, representing a consensus of currently available climate change predictions.

In order to clearly describe how habitat suitability for each disease is predicted to change over time under climate change, I created habitat suitability change maps. Each individual climate projection was converted to a binary layer using a habitat suitability cutoff of 0.8 (on a scale from 0, unsuitable habitat, to 1, most suitable habitat) where values less than 0.8 were conservatively categorized as pathogen absence, and values greater than 0.8 were categorized as pathogen presence. Next, projections from present through to 2080 under each RCP were combined to identify areas of overall increasing and decreasing habitat suitability over time using ArcMap 10.1 Spatial Analyst (ESRI, 2013). This approach was chosen to clearly identify areas of habitat suitability change since it can be very difficult to identify changes from one model to the next when comparing projections across multiple RCPs and years.

Results

Present models of SIVcpz and SFVcpz

All models that performed poorly (ROC < 0.6 and/or TSS < 0) were rejected, and remaining models were combined to create an ensemble projection weighted by model performance scores (ROC and TSS). Poorly performing models included: Generalized Additive Model (GAM), Generalized Linear Model (GLM), and Artificial Neural Networks (ANN) for both SIV*cpz*Ptt and SIV*cpz*Pts and GAM, Generalized Boosting Model (GBM), and Surface Range Envelope (SRE) for SFV*cpz*Ptt and

SFV*cpz*Pts. Final model ensembles are shown in Figure 2 using a logarithmic scale ranging from 0, corresponding to low probability of occurrence (cooler colors), to 1, corresponding to high probability of occurrence (warmer colors). Figure 2A shows ensembles for SIV*cpz*Ptt and SIV*cpz*Pts; since no presence samples were collected from *P. t. ellioti* populations for SIV*cpz*, a model could not be created. Figure 2B shows ensembles for SFV*cpz*Pte, SFV*cpz*Ptt, and SFV*cpz*Pts.

Niche overlap tests of ensemble model projections suggest that SIV*cpz* and SFV*cpz* are separated both geographically and ecologically. Models of SIV*cpz* are driven by precipitation seasonality, precipitation of the wettest month, and mean diurnal range contributing a collective 67%. Models of SFV*cpz* are driven by annual precipitation, precipitation of the wettest quarter, and temperature seasonality contributing a collective 71%. Ensemble models and environmental variable response curves show that presence of SIV*cpz*Ptt and SIV*cpz*Pts are highly associated with regions of high precipitation and low precipitations seasonality, while presence of SFV*cpz*Pte, SFV*cpz*Ptt, and SFV*cpz*Pts are highly associated with regions of high temperature, average-low precipitation, and with hot dry seasons (Appendix 3.1).

Niche divergence testing between SIVcpz and SFVcpz

Within chimpanzee subspecies, ensemble projections of SIV and SFV were compared using both a Pearson Correlation test and a niche comparison test using methods inspired by ENMtools (Warren et al., 2010). Table 1 shows values for the Schoener's *D* (Warren et al., 2010) and the *I* test statistic (Warren et al., 2008) from

the pairwise niche comparison tests between SIV*cpz* and SFV*cpz* within *P. t. troglodytes* and *P. t. schweinfurthii*. Results suggest that distributions of SIV*cpz*Ptt and SFV*cpz*Ptt are highly divergent from each other using both test statistics ($p < 0.001$), and according to the Pearson correlation test, they have low correlations ($R = 0.134$, $p < 0.001$). Distributions of SIV*cpz*Pts and SFV*cpz*Pts were also highly divergent from each other using both Schoener's *D* and *I* ($p < 0.001$), and Pearson correlation tests showed low correlation ($R = 0.215$, $p < 0.001$). These results reflect those from Locatelli et al. (2016), showing very little overlap between predicted ranges of SIV*cpz* and SFV*cpz* occurrence in chimpanzees.

Future models of SIVcpz and SFVcpz

Present models of SFV*cpz* and SIV*cpz* were projected under climate change scenarios from the TERRA6-AR5 multi-model ensemble (Chapter 5)(Sesink Clee, In prep.) using Biomod2 (Thuiller et al., 2013) and Biomod2EZ (Chapter 6)(Sesink Clee et al., In prep.). Changes in habitat suitability can be difficult to observe when faced with ecological niche model projections for five pathogens (SIV*cpz*Ptt, SIV*cpz*Pts, SFV*cpz*Pte, SFV*cpz*Ptt, and SFV*cpz*Pts), across four representative concentration pathways (RCP 2.6, 4.5, 6.0, and 8.5), and four time periods (present, 2030, 2050, and 2080). In order to synthesize these 80 projections to clarify overall trends, final ensemble models for each virus were combined across RCPs and years to create maps that estimate habitat suitability change for SIV*cpz* and SFV*cpz*. Each individual model projection was compared to the previous projection in the time series (Appendix 3.2-3.6) to calculate if the habitat is estimated to become more or

less suitable. These resulting binary maps show increasing habitat suitability (green) or decreasing habitat suitability (red) (Figure 3). Habitat suitability change maps were compared to each other using a Pearson correlation test, which showed little overlap between SIV*cpz*Ptt and SFV*cpz*Ptt ($R = 0.201$, $p < 0.001$) and between SIVcpzPts and SFV*cpz*Pts ($R = 0.238$, $p<0.001$).

Discussion

A recent study identified potential divergence between distributions of Simian Immunodeficiency Virus (SIV*cpz*Ptt) and Simian Foamy Virus (SFV*cpz*Pte and SFV*cpz*Ptt) occurrence in chimpanzees across Cameroon (Locatelli et al., 2016). Ecological niche models revealed very little geographic overlap of SFV*cpz* and SIV*cpz* occurrence in this region, and *P. t. ellioti* showed no signs of harboring SIV. Locatelli et al. (2016), however, included only a small portion of the full range of *P. t. troglodytes* and used samples only from Cameroon and Nigeria. Because this study included only a small portion of *P. t. troglodytes* range, the resulting models may have undersampled the full range of ecological factors that define the niches for both SFV and SIV in chimpanzees. The present study included samples from the full ranges of *P. t. ellioti*, *P. t. troglodytes*, and *P. t. schweinfurthii* and used a more robust ensemble modeling technique. Overall, niche divergence appears to be a general feature of the distributions of SIV*cpz* and SFV*cpz* in both *P. t. troglodytes* and *P. t. schweinfurthii*; SIVcpz and SFVcpz have an inverse relationship with one another across equatorial Africa, and environmental variation appears to occupy an important role in determining their distributions.

The SIV +/- boundary at the Sanaga River

Although previous studies have not detected SIV*cpz* in *P. t. ellioti*, none have yet been published along with ecological niche models that encompass the entire range of both subspecies at the SIV*cpz* +/- boundary, which has the potential to describe regions that are undersampled. This SIV*cpz* +/- boundary at the Sanaga River in Cameroon may persist for a number of reasons. One explanation could involve *P. t. ellioti* maintaining some level of SIV resistance that prevents them from becoming infected even though there is evidence for ongoing migration between *P. t. ellioti* and *P. t. troglodytes* (Mitchell et al., 2015b). In *P. t. verus*, reduced genetic diversity at the 5 CCR5 locus suggests a selective sweep that may be due to a response from the outbreak of a SIV-like ancestor (Wooding et al., 2005). It appears that this selective sweep driven by retrovirus infections may have occurred before the separation of chimpanzee subspecies over 2 million years ago (De Groot et al., 2008; De Groot et al., 2010), leading to the difference observed today in viral presence (Macfie et al., 2009). In humans some Caucasians have a mutant form of the co-receptor CCR-5 that greatly reduces the chances of HIV infection and/or proliferation of the virus (Samson et al., 1996) and a similar mutation may exist in *P. t. ellioti* that keeps them from becoming infected with SIV*cpz*. Geographic separation from *P. t. troglodytes*, although only partially complete, may have allowed this mutation to proliferate throughout populations of *P. t. ellioti* and result in a subspecies-wide resistance to SIV*cpz* infection.

Even partial resistance to a virus can keep it from infecting an entire population. This can occur if a population was previously infected with a pathogen or disease,

leading to a bottleneck where surviving individuals have some level of resistance. Even if this resulting population had a mixture of both resistant and non-resistant individuals, the concept of 'herd immunity' may come into play. The term 'herd immunity' describes a population that has a large proportion of immune or resistant individuals that protect individuals that lack resistance (Fine, 1993). Although herd immunity is often used in determining resistance to a pathogen by vaccinating a certain percentage of the population, this concept can be applied broadly to wild populations of animals with acquired immunity or resistance to a given pathogen. If a population of chimpanzees has a large proportion of individuals that are resistant to SIV infections due to a population bottleneck or selective sweep, the lifecycle and spread of SIV could be disrupted, slowed, or stopped completely. The potential for herd immunity is also increased when it comes to pathogens that are transferred directly between individuals without the need for an intermediate host (Lyles et al., 1993). Herd immunity may help explain SIV absence in *P. t. ellioti* since there are low levels of migration with neighboring *P. t. troglodytes* (Mitchell et al., 2015a), leading to fewer opportunities for SIV to be introduced.

Another potential explanation for the lack of evidence for suitable habitat of SIV*cpz* north of the Sanaga River could be that coinfection of SFV*cpz*Pte and SIV*cpz* is lethal. A study on rhesus macaques found evidence that pre-existing SFV infections increased the virulence of new SIV infections, causing them to lead to mortality at a much faster rate than by SIV alone (Choudhary et al., 2013). Data collected from previous studies of viral presence in chimpanzees did not show strong evidence for coinfected *P. t. ellioti* individuals (Liu et al., 2008), which could be explained by a

coinfection lethality hypothesis (Choudhary et al., 2013). Since SFV*cpz* is well established and widespread in chimpanzees, including *P. t. ellioti*, the introduction of SIV*cpz* could lead to increased virulence, decreasing its ability to proliferate in a population and making it difficult to detect in collected fecal samples.

Niche differentiation between SIVcpz and SFVcpz within P. t. troglodytes and P. t. schweinfurthii

Aside from the SIV +/- boundary at the Sanaga River between *P. t. ellioti* and *P. t. troglodytes*, niche differentiation between SIV*cpz* and SFV*cpz* within *P. t. troglodytes* and *P. t. schweinfurthii* is particularly interesting since these pathogens occur within the same subspecies. The lack of overlap between SIV*cpz* and SFV*cpz* identified by Locatelli et al. (2016) appears to persist across the ranges of *P. t. troglodytes* and *P. t. schweinfurthii*. Unlike the boundary at the Sanaga River between *P. t. ellioti* and *P. t. troglodytes*, it is more difficult to explain the apparent geographic division between pockets of SIV*cpz* and SFV*cpz* occurrence in chimpanzee subspecies that have been found to be infected with both. Under present conditions, models of SIV*cpz* and SFV*cpz* presence in *P. t. troglodytes* and *P. t. schweinfurthii* show very little overlap both geographically and in environmental niche space. This trend may be caused by a variety of different mechanisms including: (1) viral competition, (2) localized immunity, (3) separation by geographic barriers, and (4) ecological/environmental factors.

Viral competition has been well studied using assays involving closely related viral strains in laboratory settings (Quiñones-Mateu et al., 2000; Zhang et al., 2000). In

some cases, the introduction of different strains of similar viruses can result in competition within hosts that leads to the transmission reduction in one and increase in the other (Pepin et al., 2008). In cases where viral strains are closely related, they may be competing for resources that eventually lead to one becoming more prevalent. Although they target a variety of tissues and cell types, SIV and SFV overlap with the infection of CD4+ lymphocytes (Bailes et al., 2003; Murray et al., 2006; Choudhary et al., 2013). In addition to cells involved in immune response such as helper T cells and macrophages (Clapham et al., 1991), SFVs also target and infect a wider variety of tissues to form syncytia (Linial, 1999). The exact mechanisms that these viruses undergo within infected individuals are not fully known and further research may uncover more detailed interactions between them within coinfected hosts.

Localized immunity to either SIV or SFV is another potential explanation for the lack of geographic overlap between them within chimpanzees. As previously discussed in terms of the absence of SIV in *P. t. ellioti*, population-wide events may have resulted in areas with differential levels of viral immunity or resistance that describe their distributions today. This pattern could be explained by geographic separation from natural barriers such as rivers and impassible terrain that could theoretically divide populations of chimpanzees, restricting migration and allowing immunity to build or simply keep the two viruses from interacting at present time. In this case, boundaries between pockets of SIV*cpz* and SFV*cpz* presence do not entirely coincide with geographic boundaries. For example, at the Congo River which divides *P. t. troglodytes* and *P. t. schweinfurthii* which may limit gene flow,

trends of SIV presence and SFV absence appear to stretch across the subspecies boundaries unimpeded.

Just as the distribution of host species can be driven by environmental and climatic factors, microbiota and pathogens can be affected in similar ways (Larsen et al., 2012; Gilbert et al., 2014). External conditions that best suit the host taxa can affect the internal environment that pathogens require to survive, thereby shaping the geographic distribution of the resulting disease (James et al., 2015). For diseases that include a vector for transmission during the pathogen lifecycle, the precise environmental conditions required for both the host and the vector taxa must coincide in order for the pathogen to be able to persist and spread effectively. Although the lifecycle of SIV and SFV do not involve vector taxa, they still rely on their primate hosts to survive, and thus, poor environmental conditions for these hosts may result in the absence of the disease. This does not mean that suitable habitat for chimpanzees in Central Africa is always suitable for these two retroviruses, since changes in the external environment, even when small, have the potential to alter the internal environment of the host and affect the presence of the pathogens that they harbor (Duncan et al., 2011; Goedknegt et al., 2015).

Lastly, chimpanzee behavior and viral transmission routes may contribute to the apparent niche separation between SIV*cpz* and SFV*cpz* occurrence (Locatelli et al., 2016). In chimpanzees, SIV and SFV appear to transmit from one individual to another in different ways: SIV appears to spread horizontally primarily by sexual contact with potential to spread vertically from mother to offspring (Apetrei et al.,

2004), while SFV appears to spread mainly through aggressive behaviors between adult individuals such as bites or saliva coming in contact with open wounds (Murray et al., 2006).

SIVcpz and SFVcpz distributions under climate change

Regions that, on average, increased in suitability over time across each scenario for one pathogen, appear to be inversely correlated with regions that decreased in suitability for the other pathogen. This pattern further supports the hypothesis that environmental and climatic conditions, at least in part, shape the differential distributions of SFV*cpz* and SIV*cpz* within *P. t. troglodytes* and *P. t. schweinfurthii* in central Africa. There may be cases where two distributions appear to be completely divergent under present conditions, but under climate change, the niches overlap more and show coalescence. This does not appear to be the case with SIV*cpz* and SFV*cpz* infecting *P. t. troglodytes* and *P. t. schweinfurthii*, which show inverse geographic niche shifts over time and large differences in ecological niches.

Conclusion

Niche divergence and persistent geographic separation between infectious diseases within the host taxa can be the result of a number of different scenarios, but it is difficult to determine which is the most plausible. The potential explanations of viral competition, geographic separation by physical barriers or host niche requirements, lethal co-infections, or some combination of these may play a

role in the apparent niche and geographic divergence between SIV*cpz* and SFV*cpz*. Interactions between these pathogens need to be investigated further to properly identify the fine-scale causative agents of divergence. Although the ecological niches of SIV*cpz* and SFV*cpz* show little geographic and niche overlap, there may be other pressures besides climatic and environmental conditions that contribute to the observed niche divergence. Future studies investigating SFV and SIV in other African primates, such as gorillas, may provide useful insights about the patterns observed in chimpanzees. Additional research investigating the interaction between SIVs and SFVs to identify potential viral interactions and competition for resources within infected hosts may also help explain the divergence identified in this study. Recent studies have shown a detailed population genetic structure within *P. t. ellioti* in Cameroon and Nigeria attributed to environmental, climatic, and geographic variables (Mitchell et al., 2015a; Mitchell et al., 2015b; Sesink Clee et al., 2015) and similar patterns in other chimpanzee subspecies that can be infected with both SIV and SFV (*P. t. troglodytes* and *P. t. schweinfurthii*) may reveal genetically distinct populations with varying resistances to these pathogens.

Figures

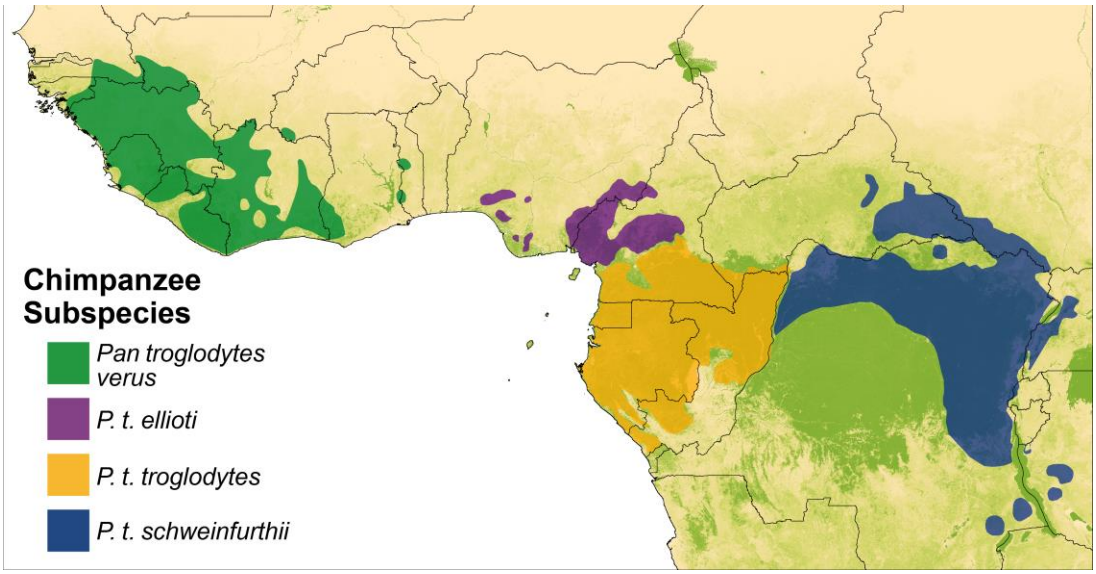

Figure 1. Ranges of chimpanzee subspecies across Africa: *Pan troglodytes verus* – Western Chimpanzee (green), *P. t. ellioti* – Nigeria-Cameroon Chimpanzee (purple), *P. t. troglodytes* – Central Chimpanzee (orange), and *P. t. schweinfurthii* – Eastern Chimpanzee (blue).

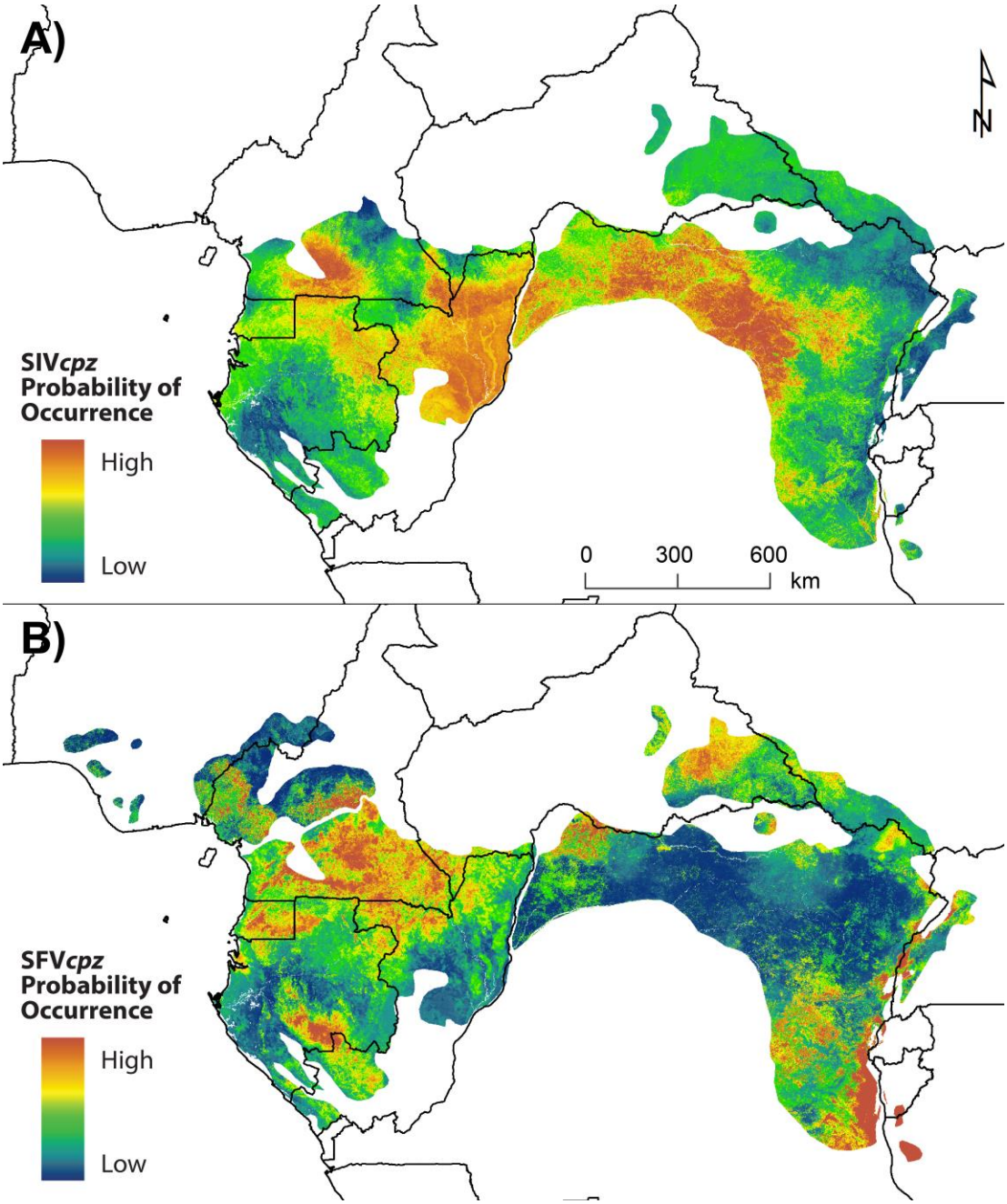

Figure 2. Projected ensemble occurrence models for (A) SIV*cpz* and (B) SFV*cpz*. Cool cooler represent low probability of occurrence and warm colors represent high probability of occurrence.

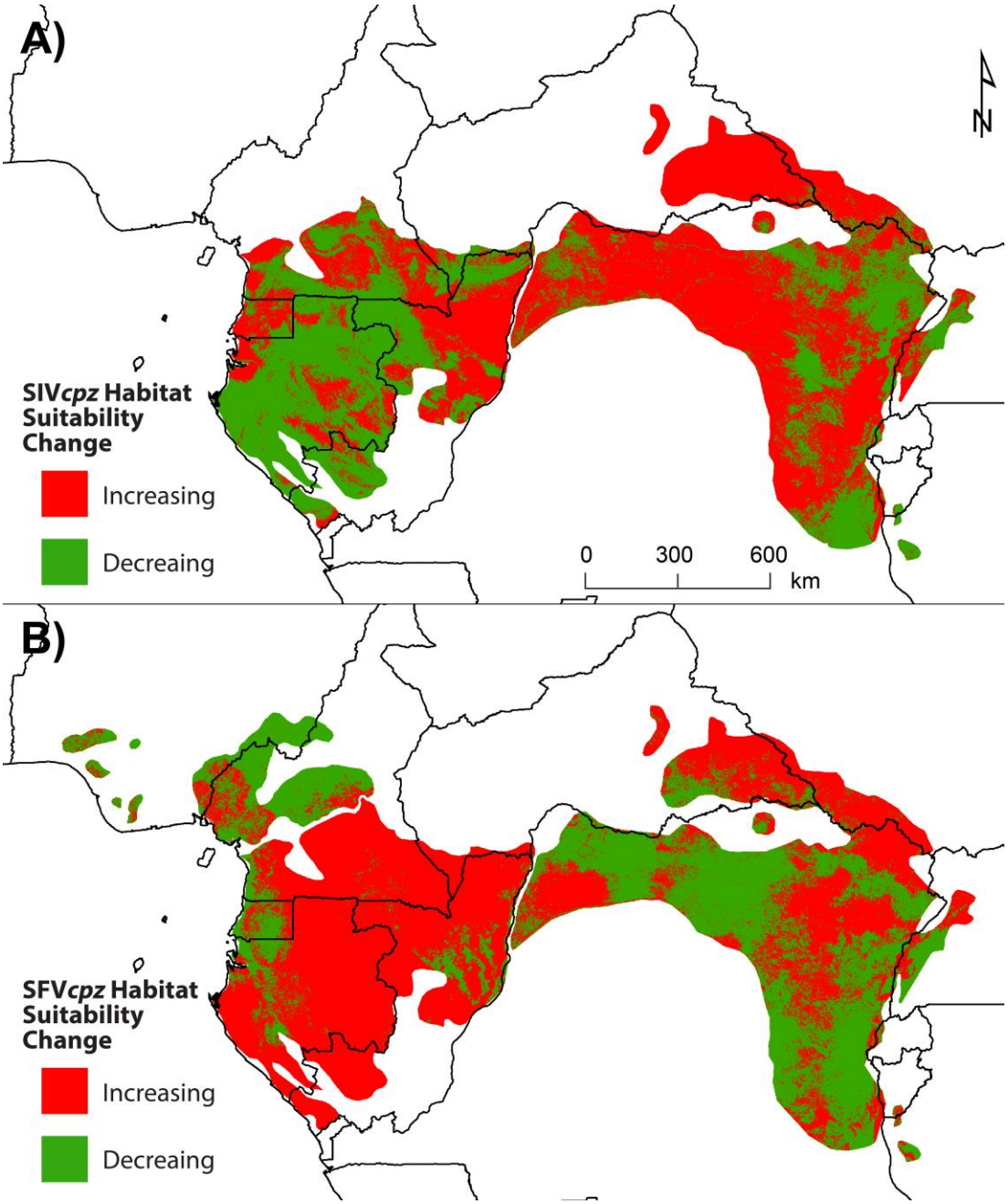

Figure 3. Habitat suitability change maps for (A) SIV*cpz* and (B) *SFVcpz* occurrence under climate change between present and 2080. Red represents decrease in habitat suitability and green represents increase in habitat suitability.

Tables

Table 1. SIV/SFV niche overlap tests for *P. t. troglodytes* and *P. t. schweinfurthii*

	Schoener's *D*				***I***				**Pearson's Correlation Coefficient**
Comparison	**Observed**	**Null Mean**	**Null SD**	***p***	**Observed**	**Null Mean**	**Null SD**	***p***	
SIV*cpz*Ptt and SFV*cpzPtt*	0.092	0.756	0.023	<0.001	0.312	0.916	0.018	<0.001	0.134
SIV*cpzPts* and SFV*cpzPts*	0.081	0.742	0.018	<0.001	0.287	0.902	0.010	<0.001	0.215

Chapter 4: Biodiversity and wildlife infectious disease occurrence: understanding the ape Ebola crisis

Abstract

The Gulf of Guinea in west Central Africa is both a biodiversity hotspot and a center of Ebola Virus Disease (EVD) outbreaks that has spread rapidly in the past decade, leading to pronounced declines in Western Lowland Gorilla (*Gorilla gorilla gorilla*) and Central Chimpanzee (*Pan troglodytes troglodytes*) populations in Gabon and Republic of the Congo. Recent research in this region suggests that frugivorous bats may be a natural reservoir for Ebola viruses, transmitting it to other mammals via partially eaten fruit. In this study, I aimed to test if EVD occurrence in chimpanzees and gorillas is associated with levels of biodiversity. There are two main hypotheses on the relationship between wildlife infectious disease occurrence and biodiversity: the Dilution Effect and the Amplification Effect. The Dilution Effect hypothesis states that biodiversity and disease presence are inversely related, while the Amplification Effect hypothesis states that biodiversity and disease presence are directly related.

Using samples from previous studies detecting EVD infections in wild Central Chimpanzees (*P. t. troglodytes*) and Western Lowland Gorillas (*G. g. gorilla*), I created predictive models of EVD occurrence in chimpanzees and gorillas across their shared range in southern Cameroon, Equatorial Guinea, Gabon, and Republic of the Congo using both environmental and biodiversity predicting layers. Additional models were created using combined samples from chimpanzees and gorillas to

create an overall EVD hotspot map, which was used to quantify underlying biodiversity measures. Distribution models estimated the highest probability of occurrence in northeast Gabon and neighboring parts of Cameroon and Republic of the Congo, showing negative correlations with overall mammal species richness and primate species richness, and positive correlations with bat species richness. These results suggest support of the Dilution Effect hypothesis for primate hosts and the Amplification Effect for potential natural reservoirs. This positive correlation with bat species richness may indicate that there are multiple bat species that act as competent reservoirs for the virus, where increased bat species richness results in an increase of reservoir taxa. The Dilution Effect observed with primate species may occur due to saturating the community with low competency primate hosts, disrupting viral spread through populations. It appears that EVD occurrence cannot be entirely explained by the Dilution or Amplification Effects, and may be better explained by optimal community composition.

Introduction

Ebola virus outbreaks in humans and non-human primates

Ebola viruses belong to the family Filoviridae, genus *Ebolavirus* and currently include five different species: *Bundibugyo ebolavirus* (BDBV), *Reston ebolavirus* (RESTV), *Sudan ebolavirus* (SUDV), *Taï Forest ebolavirus* (TAFV), and *Zaire ebolavirus* (ZEBOV) (Pourrut et al., 2005). In 1976, the first species were discovered (ZEBOV and SUDV) from initial outbreaks in Zaire (now Democratic Republic of the Congo) and Sudan with fatality rates of 50-85% (World Health Organization, 1978;

Heymann et al., 1980). Initial studies on these early outbreaks in humans suggested that they likely each originated from single transmission events from an unknown zoonotic source, followed by repeated transmissions between humans via contact with bodily fluids of infected individuals (Pourrut et al., 2005; Leroy et al., 2007). More recently, since the early 2000s, there have been more than 10 outbreaks in central and west Africa that have collectively infected more humans than all previous Ebola outbreaks combined, with more than 28,000 human cases in total (World Health Organization, 2016).

The origins of Human Ebola virus outbreaks and its impacts on human populations have been well studied from several perspectives including transmission between people and public health aspects for mitigation (Pourrut et al., 2005; Muyembe-Tamfum et al., 2012; Althaus, 2014; Annette et al., 2015; Agnandji et al., 2016). Although data remain sparse about Ebola's impact on wildlife, the virus appears to infect several non-human primate species in Africa (Formenty et al., 1999; Leroy et al., 2004a; Lahm et al., 2007; Huffman et al., 2009). From 1994 through 2005, Ebola outbreaks in Gabon and Republic of the Congo caused pronounced declines in chimpanzee and gorilla populations, with mortality of over 90% of individuals in these communities, which have yet to completely recover (Leroy et al., 2005; Bermejo et al., 2006). Over the course of 3 months, 8 groups of gorillas in Lossi Gorilla Sanctuary consisting of 143 individuals, disappeared completely, suggesting that Ebola virus outbreaks in apes spread rapidly, leading to high mortality rates in a short amount of time (Leroy et al., 2004b). Multiple strains of Ebola virus were detected in many of the carcasses, suggesting that they became infected by contact

with a natural reservoir, and the virus strains then swept through their communities from contact with infected individuals (Leroy et al., 2004b).

Although the natural reservoir for Ebola viruses is debated, there is growing evidence suggesting that fruit bats (Suborder Megachiroptera) in Africa are a likely natural host (Leroy et al., 2005; Huffman et al., 2009; David et al., 2012; Leendertz et al., 2016a). Following an outbreak of ZEBOV in 1996, experimental inoculations of the virus in local wildlife showed that it could replicate successfully in fruit bats while they remained asymptomatic (Swanepoel et al., 1996). Later in 2007, following an outbreak of the very closely related Marburg Virus in miners in Uganda, surveys of the mines led to the discovery of bats that tested positive for the virus (Towner et al., 2009). In an area of repeated ZEBOV outbreaks in humans and wildlife in Gabon and Republic of the Congo, researchers sampled a variety of bats and identified three species that tested positive for IgG antibodies specific to ZEBOV or had RNA sequences for ZEBOV (Leroy et al., 2005). More recently, comprehensive studies covering Ghana, Nigeria, Gabon, Republic of the Congo, and Democratic Republic of the Congo found more than 3,000 fruit bats in total that tested positive for ZEBOV or other Ebola viruses without signs of symptomatic infections (Pourrut et al., 2005; Hayman et al., 2010; David et al., 2012). The transmission of the virus from bat reservoirs to other wildlife is proposed to occur via the consumption of fruit that has been partially eaten by bats (Leroy et al., 2005; Alexander et al., 2015) since it is transmitted through contact with bodily fluids, including saliva (Leroy et al., 2005). If fruit bats can act as natural reservoirs of

Ebola viruses, transmission frequency may increase during dry seasons when fruit scarcity can lead to increased contact between different taxa (Huffman et al., 2009).

Biodiversity and its effect on disease presence in wildlife

Several studies have predicted the distributions of infectious diseases in wildlife, most of which have focused on how abiotic environmental variables contribute to disease occurrence (Daszak et al., 2013; Atkinson et al., 2014; Raffel et al., 2015). Infection pathways in diseases are often complex, however, and involve multiple taxa (Leroy et al., 2007; Viana et al., 2014; Loh et al., 2015; Kamath et al., 2016). The contributions of biotic variables and species interactions to disease distributions remain poorly characterized, and likely underpin the current lack of understanding about the natural history of Ebola and episodic outbreaks in human and ape populations (Alexander et al., 2015; Gale et al., 2016; Leendertz et al., 2016a). Disease distribution models that include ecological measures of diversity can better characterize the biotic communities that these diseases, their hosts, and natural reservoirs inhabit than using abiotic factors alone (Peterson, 2006b; Cross et al., 2009).

Biodiversity is a broad term that generally refers to the variety of life present across a given area, ranging from small locales and ecosystems to continental or global extents (Heywood et al., 1995). Changes in species richness appear to be associated with the distribution and occurrence of wildlife infectious diseases in many cases, and increasing evidence suggests that areas with reduced biodiversity are associated with increased disease risk (Roche et al., 2012; Johnson et al., 2013b).

Two mechanisms have been proposed to explain this relationship are (1) the Dilution Effect and (2) the Amplification Effect (Jones et al., 2008). The Dilution Effect hypothesis predicts an inverse correlation between biodiversity and disease risk, whereas the Amplification Effect hypothesis predicts that biodiversity is directly correlated with disease risk (Figure 1)(Ostfeld et al., 2000; Keesing et al., 2006; Jones et al., 2008). These two competing hypotheses are best viewed as extremes along a continuum in the relationship between diversity and disease occurrence where disease risk can be a function of several variables, including but not limited to the distribution of the pathogen, the range of the host, and the level of exposure of the host to the pathogen (Pagán et al., 2012; Johnson et al., 2013a; Elena et al., 2014).

The Dilution Effect hypothesis (Ostfeld et al., 2000; Keesing et al., 2006; Keesing et al., 2010) proposes that areas of high biodiversity are less likely to have high infectious disease occurrence, and decreasing biodiversity results in increased disease risk. Under this scenario, increased biodiversity can result in more incompetent hosts that are inefficient or unable to pass the pathogen on to new hosts, and therefore 'dilute' disease occurrence in species rich environments by interruption of pathogen transmission. The most noted example of the Dilution Effect involves the state of Lyme disease in North America (Ostfeld et al., 2000; Keesing et al., 2006). In this region, tick larvae prefer to take blood meals from the white-footed mouse (*Peromyscus leucopus*), which is an incredibly efficient reservoir for transmitting the spirochete bacterium responsible for Lyme disease, *Borrelia burgdorferi*, to ticks at rates of over 90% (Piesman, 1993). After developing into

nymphs over the winter, ticks are much less particular about their next blood meal, and often opportunistically select deer or humans, effectively spreading *B. burgdorferi* that can cause Lyme disease (Piesman et al., 1994; Van Buskirk et al., 1995; Steere et al., 2004). This Dilution Effect hypothesis has since been proposed to play a role in the distribution of several other disease-causing pathogens, including West Nile Virus and amphibian fungal infections (Logiudice et al., 2003; Ezenwa et al., 2006; Keesing et al., 2006; Swaddle et al., 2008; Johnson et al., 2013b). This hypothesis suggests a situation where conservation efforts, which aim to increase biodiversity overall, could simultaneously reduce the risk of disease outbreaks in protected species and potentially the risk of disease crossover to humans (Jones et al., 2008).

The Amplification Effect hypothesis is rooted in early epidemiological research suggesting that a threshold of host diversity exists, under which diseases would not be able to fully invade (Kermack et al., 1927). The Amplification Effect may occur when host diversity is high, increasing interactions between host taxa that could lead to the spreading of a disease throughout a population under the assumption that there are multiple taxa that can act as competent disease hosts (Hamer, 1906; Black, 1966). This mechanism may occur in a community with high species richness and evenness with more generalist pathogens that have many suitable hosts. Studies supporting the Amplification Effect in natural populations are sparse compared to the Dilution Effect (Salkeld et al., 2013). The Amplification Effect has been identified in pathogens that exist in aquatic ecosystems, where low host density can hinder pathogen transmission and spread, leading to overall declines in

disease occurrence (Hamer, 1906; Kermack et al., 1927; Black, 1966; Lafferty et al., 1999). The Amplification Effect has also been proposed to be important in in the spread of rabies by bats (De Thoisy et al., 2016).

The Dilution and Amplification Effect hypotheses go beyond a simple correlation between biodiversity and disease occurrence, and raise an important question: does biodiversity loss affect disease risk to a region? According to the Dilution Effect hypothesis, biodiversity loss in a community could result in increased the occurrence of diseases such as Lyme disease (Ostfeld et al., 2000) and West Nile Virus (Swaddle et al., 2008), increasing disease risk to both wildlife and humans. More research of wildlife infectious diseases is especially important in the tropics due to its high levels of overall diversity, with these regions supporting over half of all animal and plant species (Mittermeier et al., 2011), and rampant habitat loss from human expansion and exploitation of natural resources (Mabogunje, 1995; Atyi et al., 2013; Edwards et al., 2014; Un Desa, 2015). The tropics also appear to be a more prominent source of infectious disease outbreaks than in temperate regions (Osborne, 2000). Better understanding of the dynamics between wildlife infectious diseases and biodiversity are important in the tropics, since their interactions are not yet entirely understood (Salkeld et al., 2013).

Study region and research aims

The area of interest for this study lies at the intersection of the Gulf of Guinea biodiversity hotspot with the Congo Basin in western equatorial Africa, encompassing parts of Cameroon, Equatorial Guinea, Gabon, Central African

Republic, and Republic of the Congo (Myers et al., 2000; Mittermeier et al., 2011). This megadiverse region supports a large range of taxa including, but not limited to, numerous species of insects, amphibians, reptiles, birds, small mammals, and primates (Jones, 1994; Oates et al., 2004). Forests in this region are especially important for primates, and are home to nearly 30 different species, including chimpanzees (*Pan troglodytes*) and gorillas (*Gorilla gorilla*) (Oates et al., 2004) that have experienced repeated outbreaks of Ebola virus disease in many local populations (Huijbregts et al., 2003; Leroy et al., 2004b). Fruit bats are proposed to be the natural reservoirs responsible for the Ebola outbreaks in apes and humans (Leroy et al., 2005), but given the remoteness of the region, combined with the reclusive nature of apes, it has been difficult to identify how biodiversity affects disease risk in this tropical region and to use this information to improve understanding about Ebola outbreaks in apes. Thus, this study was designed to address two key questions: (1) Do biotic factors such as species richness and evenness play an important role in shaping the distribution of Ebola virus disease in chimpanzees and gorillas of west central Africa? and (2) If biotic factors shape Ebola occurrence in apes, does the pattern conform to expectations of the Dilution Effect, the Amplification Effect, or a more complex scenario along the disease risk continuum?

Methods

Disease occurrence data collection

To assess the relationship between the occurrence of Ebola virus and biodiversity, I first estimated the geographic distribution of the disease infecting non-human primate populations. I compiled data from previously published studies that tested wild Central Chimpanzees (*P. t. troglodytes*) and Western Lowland Gorillas (*G. g. gorilla*) for EVD (Huijbregts et al., 2003; Leroy et al., 2004b; Rouquet et al., 2005; Bermejo et al., 2006). In these studies, researchers collected fecal samples and harvested tissue from deceased individuals in the wild, and tested them for the presence of Ebola viruses (Huijbregts et al., 2003; Leroy et al., 2004b; Rouquet et al., 2005; Bermejo et al., 2006). In total, this dataset consisted of 89 presence and absence points for EVD in chimpanzees and gorillas, and includes samples from recent outbreaks in Gabon and Republic of the Congo (Huijbregts et al., 2003; Leroy et al., 2004b). By combining data from several studies, I was able to assemble a widespread presence/absence dataset of EVD occurrences in chimpanzees and gorillas while avoiding clumped sampling that could introduce modeling biases in subsequent analyses (Peterson et al., 2011).

Environmental and biodiversity predicting layers

In addition to presence and absence localities used to create distribution models, map layers that quantitatively describe the study area's habitat are also required. In this study, disease occurrence models were created using both environmental and biodiversity layers to assess how biodiversity measures could be

used to predict disease occurrence and to determine how they might be correlated with estimated disease presence. Environmental layers used in this study included measures of temperature, precipitation, seasonality (Hijmans et al., 2005), topography (Farr et al., 2007), and vegetation (Long et al., 2001; Freedman et al., 2010; Dimiceli et al., 2011) (Table 1). These layers were used to create initial niche models for EVD occurrence in chimpanzees and gorillas separately across their shared range before introducing biodiversity predictors. Biodiversity layers were assembled from a variety of sources and chosen to comprehensively describe the diversity of host and potentially related taxa to EVD in Central Africa (Oates et al., 2004; Ceballos et al., 2006; Jenkins et al., 2013b; World Wildlife Foundation, 2016) and include detailed measures for specific taxonomic orders of mammals and level of threat to extinction for mammals (Table 1). To reduce redundancies between predicting layers (environmental and biodiversity inclusive), pairwise Pearson correlation tests (Pearson, 1895) were preformed using ENMtools (Warren et al., 2010) to identify groups of variables that showed significantly similar trends across the study region. For groups of layers that were highly correlated ($R < -0.8$ or $R > 0.8$), a single layer was chosen that contributed most to preliminary distribution models created using Biomod2 (Thuiller et al., 2013).

In addition to using detailed measures of biodiversity by taxonomic order, I created broad species richness and evenness measures for all mammals combined across the study region (Appendix 4.1). First, measures of species richness were collected from publically available sources for antelopes, shrews, bats, large mammals, and primates (Table 1) and were reclassified to a spatial resolution of 30 arcseconds

(about 1km^2) to match the resolution of distribution models for later comparisons. Layers were converted when necessary to represent species counts at each grid cell, and summed to create an overall species richness map for mammals in this region. Shannon's diversity index scores (Magurran, 1988) were determined for each taxonomic order of mammals included in the species richness layer and were combined to calculate evenness at each grid cell across the study region.

Modeling procedure and biodiversity correlation analysis

I created ecological niche models for Ebola virus disease occurrence in chimpanzees, gorillas, and both combined (Appendix 4.2-4.7) with environmental predicting layers using Biomod2 (Thuiller et al., 2013) implemented in the R statistical framework (R Development Core Team, 2016) with additional visualization using Biomod2EZ (Chapter 6)(Sesink Clee et al., In prep.). Biomod2 represents a leap forward in ecological niche modeling techniques by allowing users to run up to 11 different types of niche models using the same set of presence/absence localities and predicting map layers (Thuiller et al., 2013). Users can control the individual parameters for each model and create a final ensemble model that is weighted by individual model performance scores. Ensemble models were created weighted by receiver operating characteristic (ROC) (Hanley et al., 1982) and true skill statistic performance scores (TSS) (Allouche et al., 2006) from 10 replicates of 11 different modeling techniques (110 individual models in total) using only environmental predicting factors for each disease/host pair to predict the distributions of EVD. Individual variable contributions were calculated for each

model using Biomod2 (Thuiller et al., 2013) and summarized by applying the same weighting scheme that was used to create the ensemble model.

Final ensemble models of EVD occurrence in great apes as a function of environmental predictors were tested for geographic overlap and correlations with overall mammal species richness, mammal evenness, and detailed species richness measures by taxonomic order across the study region using pairwise Pearson correlation tests (Pearson, 1895) implemented in ENMtools (Warren et al., 2010). Additional models were created for EVD occurrence in Central Chimpanzees, Western Lowland Gorillas, and both combined using only biodiversity predicting layers with the original presence/absence locality datasets (Appendix 4.5-4.7). These ensemble models were weighted by individual true skill statistic performance scores (Allouche et al., 2006) and were projected across the study region. Models for EVD occurrence in chimpanzees and gorillas using environmental predicting layers were tested for geographic overlap with those created using only biodiversity measures by Schoener's *D* (Schoener, 1968), *I* test statistic (Warren et al., 2008), and pairwise Pearson Correlation tests (Pearson, 1895) implemented in ENMtools (Warren et al., 2010) to test biodiversity measures for their ability to accurately predict disease occurrence. Lastly, ensemble model created using combined occurrence data from chimpanzees and gorillas was rescaled to a range from 0 to 1 and, using a conservative threshold of 0.8, I determined the core of highest overall probability of EVD occurrence in chimpanzees and gorillas within the study region.

In order to quantify how biodiversity indices are associated with this EVD hotspot for chimpanzees and gorillas, I focused on biodiversity factors that were important contributors to original models, and quantified them within the core of estimated EVD occurrence using zonal statistics in ArcMap 10.1 (ESRI, 2013). Next, to assess how biodiversity differs within and outside the EVD hotspot, I randomly plotted 10,000 points across the study region using ArcMap 10.1 (ESRI, 2013) and categorized them as pseudo-presence or absence points depending on their location within or outside the hotspot. These new pseudo-presence and absence points were used to create a final ensemble model with Biomod2 (Thuiller et al., 2013). Ensemble variable contribution was determined by applying the same weighting scheme used to create the ensemble model across variable contributions from individual runs along with variable response curves were created using Maxent (Phillips et al., 2006).

Results

Identifying associations between EVD occurrence and biodiversity

Models of EDV occurrence created using biodiversity measures showed a high degree geographic overlap and correlation with corresponding models created using environmental predictors for Central Chimpanzees (D = 0.71, I = 0.91, Pearson's R = 0.63, $p < 0.05$), Western Lowland Gorillas (D = 0.68, I = 0.87, Pearson's R = 0.62, $p < 0.05$), and combined across both hosts (D = 0.61, I = 0.79, Pearson's R = 0.54, $p < 0.05$) (Figure 2). Each of the three ensemble models revealed top-contributing biodiversity measures for describing EDV distributions across the

study region (Table 2). Bat species richness contributed most out of all of the included biodiversity measures in predicting the distributions for each models (> 23.85%), followed by overall mammal species richness, rodent species richness, and even-toed ungulate species richness (Table 2).

EVD occurrence models using environmental and biodiversity predictors

Individual presence ensemble models were created and projected using environmental predictors for EVD infecting Central Chimpanzees (*P. t. troglodytes*), Western Lowland Gorillas (*G. g. gorilla*), and combined chimpanzees and gorillas (Figure 3 & Table 2). These models each represent the consensus from 10 replicates of 11 different distribution modeling techniques weighted by their individual performance scores (Appendix 4.2-4.4). Disease presence distributions were then tested for correlations with important biodiversity measures identified by secondary models. EVD occurrence projections for chimpanzees and gorillas in this region showed slightly negative correlations with overall mammal species richness ($-0.18 > R > -0.26$, $p < 0.05$) and primate species richness ($-0.27 > R > -0.35$, $p < 0.05$), positive correlations with bat species richness ($0.36 > R > -0.43$, $p < 0.05$), and no clear correlation with remaining biodiversity measures ($-0.08 > R > 0.08$, $p < 0.05$). Overall mammal species evenness showed marginally negative correlations with EDV occurrence in gorillas ($R = -0.31$, $p < 0.05$), chimpanzees ($R = -0.22$, $p < 0.05$), and gorillas and chimpanzees combined ($R = -0.18$, $p < 0.05$).

Quantifying biodiversity within regions of highest EVD occurrence in chimpanzees and gorillas

Next, a presence threshold of 0.8 was applied to ensemble projections created using environmental predictors to identify regions of highest estimated probability of EVD occurrence. Additional distribution models were created with randomly plotted points to quantify differences between areas within and outside of the core of highest probability of EVD occurrence. Zonal statistics were also calculated using ArcMap 10.1 (ESRI, 2013) to quantify the composition of top-contributing biodiversity measures within core EVD occurrence regions. Core regions of high probability of EVD occurrence in chimpanzees and gorillas showed similar trends in underlying biodiversity measures. These regions are characterized by low overall mammal species richness, primate species richness, and overall mammal evenness, average species richness for carnivores, small mammals, even-toed ungulates, and rodents, and high bat species richness when compared to ranges for the entire study area. Variable response curves produced using Maxent (Phillips et al., 2006) showed that the highest increase in model gain occurs when bat species richness is between 40 and 54 (the maximum within this study region) (Figure 4). Zonal statistics and hotspot ensemble models showed that regions of highest estimated probability of EVD occurrence in chimpanzees and gorillas both overlap with the 90th percentile of bat species richness across the study region and include more than 45 difference species in total.

Discussion

Ebola virus disease occurrence and its relationship with biodiversity

Ebola virus disease outbreaks in chimpanzee and gorilla populations near human outbreaks (Leroy et al., 2004b) and in Gabon and Republic of the Congo have led to large declines in wild populations (Leroy et al., 2005; Bermejo et al., 2006). The exact origins of EVD outbreaks in non-human primates are unclear, but may be partially explained by biodiversity and community composition (Alexander et al., 2015). In this study, I modeled the probability of EVD occurrence across the shared ranges of Central Chimpanzees (*P. t. troglodytes*) and Western Lowland Gorillas (*G. g. gorilla*) using known localities of disease presence and absence and both environmental and biodiversity predicting variables (Figures 2 and 3). Although the distribution of this disease differs slightly between chimpanzees and gorillas, which may be due to host habitat use, they share a core region of highest estimated probability of occurrence in northeast Gabon and neighboring Republic of the Congo.

Models produced using biodiversity predictors and composition in regions of highest estimated probability of occurrence, showed slightly inverse relationships with overall mammal species richness and primate species richness. Regions with the highest EVD occurrence in chimpanzees and gorillas are characterized by relatively low mammal and primate species richness. The inverse relationship between EVD occurrence in apes and primate species richness suggests that areas of diversity may provide a barrier to disease proliferation, which is hypothesized to occur under different possible conditions. First, increased primate species richness

may saturate the community with low competency primate hosts, limiting exposure and disease spread overall. Secondly, high primate species richness may increase interspecific competition for fruits and favor consumption of more non-fruit fallback foods such as bark, leaves, flowers, and insects (Harrison et al., 2011), which would reduce contact with partially eaten fruits by bats: the putative natural reservoirs for Ebola virus (Leendertz et al., 2016a).

Conversely, it is also important to consider why high estimated EVD occurrence falls in areas where primate species richness is relatively low. The Dilution Effect is hypothesized to occur under a variety of different conditions, including when disturbance causes low competency sensitive species to decline, leaving only resilient species that tend to be more competent disease hosts (Keesing et al., 2006; Cary Institute of Ecosystem Studies, 2016). In this case, chimpanzees and gorillas are often found in secondary forests (Plumptre et al., 2003) and areas of moderate disturbance (White et al., 2001). Another study of chimpanzees and gorillas in southern Cameroon showed that population densities within the protected Dja Faunal Reserve were comparable to densities outside of the park in fringe habitats near logging sites (Dupain et al., 2004). This apparent Dilution Effect with EVD in apes could be used to assess risk to wildlife communities based on recent habitat disturbance. Disturbances, such as increased bushmeat hunting and large logging efforts that may reduce mammal diversity in areas where biodiversity was previously high, and increase disease risk for the taxa that remain and are able to persist in moderately disturbed habitats, such as chimpanzees and gorillas. Habitat disturbance in areas where environmental models produced in this study predict

high probabilities of EVD occurrence may ultimately increase spur new outbreaks in these ape populations.

Bats as natural asymptomatic disease reservoirs

In contrast to primates and mammals, bat species richness is significantly higher in areas of known and predicted EVD occurrence in apes. Predictive models of EVD occurrence in chimpanzees and gorillas relied primarily on high bat species richness (17.89-24.78% contribution) and 90th percentile threshold layers of bat species richness across the study region encompass areas of highest estimated probability of EVD occurrence in chimpanzees and gorillas combined. These results are consistent with evidence suggesting that bats act as a natural reservoir of Ebola viruses. For example, a recent study on the zoonotic niche of Ebola viruses across Africa (Pigott et al., 2014) modeled the distributions of the three frugivorous bat species that appear to be involved in Ebola virus lifecycles (*Hypsignathus monstrosus* , *Epomops franqueti*, and *Myonycteris torquata*) whose ranges overlap in the identified hotspot EVD occurrence and known locations of outbreaks in chimpanzees and gorillas (Leroy et al., 2005; Olival et al., 2014). My models of EVD occurrence in chimpanzees and gorillas show positive correlations with bat species richness, supporting the Amplification Effect hypothesis. Assuming multiple bat species are competent reservoirs of Ebola viruses in Africa, it is reasonable to predict that high bat species richness may result in more competent reservoir species and increase susceptibility to Ebola virus occurrence and transmissions to other wildlife.

These observations may be especially important for improving understanding about host/reservoir/pathogen relationships in ape EVD. The exact roles that bats may play in the transmission of Ebola viruses is not entirely understood, but it has been found that they can harbor Ebola viruses while remaining asymptomatic, suggesting that they may act as a natural reservoir (Huffman et al., 2009; Smith et al., 2013; Banskar et al., 2016). There is ongoing debate on how bats are able to harbor high viral loads without succumbing to their effects, but possible explanations include constantly mounting aggressive immune response to clear infections, only shedding the viruses when replication rates are very high, immune response that intensifies in pulses - keeping viruses in check, or their high metabolism and body temperature may assist with viral shedding (Zhang et al., 2012; Smith et al., 2013; Schountz, 2014). Bat roosting behavior may also contribute to their impressive viral loads since they often reside in large, high-density colonies consisting of a variety of species (Kunz et al., 2005). Thus, bat colonies may act as incubators for various pathogens by keeping them circulating within local bat communities and setting the stage for cross-species transmissions of viruses with susceptible sympatric species such as chimpanzees and gorillas (Peel et al., 2013).

This growing body of evidence suggesting that frugivorous bats may play an important role as natural reservoirs for Ebola viruses in Africa, includes a large-scale sampling effort of a variety of bat species in areas where outbreaks of EVD in humans have occurred revealing presence of ZEBOV and related antibodies (Leroy et al., 2005; Huffman et al., 2009; David et al., 2012; Olival et al., 2014; Leendertz et al., 2016a). Experimental infections of Wahlberg's epauletted fruit bat

(*Epomophorus wahlbergi*), the little free-tailed bat (*Tadarida pumila*), and Angola free-tailed bat (*Mops condylurus*) with ZEBOV resulted in successful viral replication (Swanepoel et al., 1996). Another study detected Ebola virus RNA and virus-specific immunoglobulin M antibodies in hammer-headed fruit bats (*Hypsignathus monstrosus*), Franquet's epauletted bats (*Epomops franqueti*), and little collared fruit bats (*Myonycteris torquata*) collected from the wild (Leroy et al., 2005). Even with evidence that the virus can replicate and is present in some bat species, there is not yet complete confirmation that these bats shed the virus leading to transmission to other vertebrates, but the detection of ZEBOV and related antibodies suggests that they may, in the very least, play an important role in the lifecycle of the virus as an intermediate host (Leendertz et al., 2016a).

Dilution vs. Amplification Effects in EVD infecting chimpanzees and gorillas

According to the models presented in this study, the highest probability of EVD occurrence appears to fall in areas where bat species richness is high (Amplification Effect) and primate species richness is low (Dilution Effect), both of which are important for understanding ape EVD risk. In the case of ape EVD, the Amplification Effect due to bat species richness appears to be the most important predictor of the overall risk of EVD to apes of this region. However, the secondary Dilution Effect with primates and other mammals may be another key indicator for predicting EVD risk. Other factors likely to also contribute to predicting patterns of ape EVD include dispersal barriers, geographic scale, and human disturbance. For example, geographic scale may play an underappreciated role in predicting whether

the Dilution or Amplification Effect adequately explain the distribution of EVD disease, since pathogen presence appears to be overwhelmingly directly correlated with biodiversity when looking across broad geographic scales (Hudson et al., 2006; Jones et al., 2008). The Dilution Effect suggests that biodiversity is inversely related to disease occurrence in wildlife (Ostfeld et al., 2000; Keesing et al., 2006), while the Amplification Effect suggests a direct relationship (Lafferty et al., 1999; Randolph et al., 2012). The Dilution Effect continues to be identified in studies of wildlife infectious disease distributions, and if it holds true, has an added benefit that increased conservation action could simultaneously protect more wildlife from habitat loss and other pressures, reduce disease occurrence in wildlife populations, and also reduce disease risk for humans (Jones et al., 2008). However, attempting to preserve critical habitats and, in turn, the wildlife living within them, may not be sufficient action to reduce EVD occurrence due to the observed contrasting relationships with host and reservoir biodiversity.

For the case of EVD infecting non-human primates, it appears that community composition is more important in determining probability of disease occurrence than an overarching correlation with biodiversity. Community composition can affect pathogens and result in varied support for generalists or specialists (Vander Wal et al., 2014). In communities with high biodiversity, it is hypothesized that pathogens are more likely to become specialized (Woolhouse et al., 2001), while generalist pathogens are often favored in communities with low biodiversity or where host migration is present (Kurtenbach et al., 2006). Furthermore, generalist pathogens in species-poor regions may be constrained by community assemblage

and suffer from low transmission rates and be under pressure to reduce virulence to ensure that their basic reproduction rate (r0) remains greater than 1 by spreading to at least one other host individual before host mortality occurs (Lenski et al., 1994). The greater overall community composition may play a larger role in driving the occurrence of disease-causing pathogens than relationships with biodiversity measures.

Future research

Results of this study show a strong direct relationship between EVD occurrence in chimpanzees and gorillas with bat species richness across this region of western equatorial Africa. These new findings combined with recent research identifying frugivorous bats as a potential natural reservoir of Ebola viruses highlights the need for better understanding of Ebola virus reservoir and host dynamics. More research to confirm natural reservoirs of Ebola virus in Africa that include analysis of host/reservoir species compositions and abundances where disease occurrence is high, inter- and intra-specific interactions in hosts that can spread the virus within their populations, and more insight into Ebola virus lifecycles and transmissions routes in the wild are necessary to better understand the spread of this disease. Investigation into host and reservoir species behavior, demographics, and social structure may also narrow our understanding of these trends and reveal how Ebola viruses transmit from reservoirs to hosts.

Finally, results from this study along with recent research suggesting that large declines in ape populations was caused by EVD (Huijbregts et al., 2003; Leroy et al.,

2004b), high levels of bushmeat hunting across the region (Ziegler et al., 2016), and the mosaic distribution of protected areas that are almost completely surrounded by land that is actively used for logging and agricultural purposes (Laporte et al., 2007; Rayden et al., 2010), allude to the need for new conservation action plans (Warfield et al., 2014). These connections have important implications for mitigation of EVD spread within wildlife populations. By identifying areas with the highest probability of EVD occurrence in apes across this region, research efforts that are underway to develop a vaccine for EVD in wild apes (Warfield et al., 2014; Rampling et al., 2015; Ewer et al., 2016; Leendertz et al., 2016b) can determine locations for potential trials, especially where habituation projects are already established (Nunn et al., 2016).

Figures

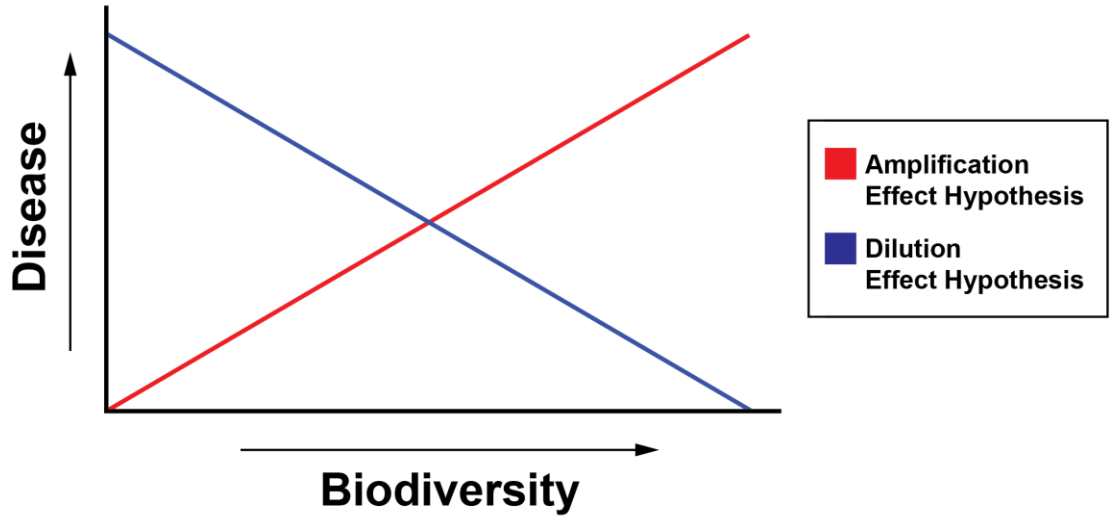

Figure 1. Diagram of the theoretical trends between disease occurrence and biodiversity under the Amplification Effect Hypothesis and the Dilution Effect Hypothesis.

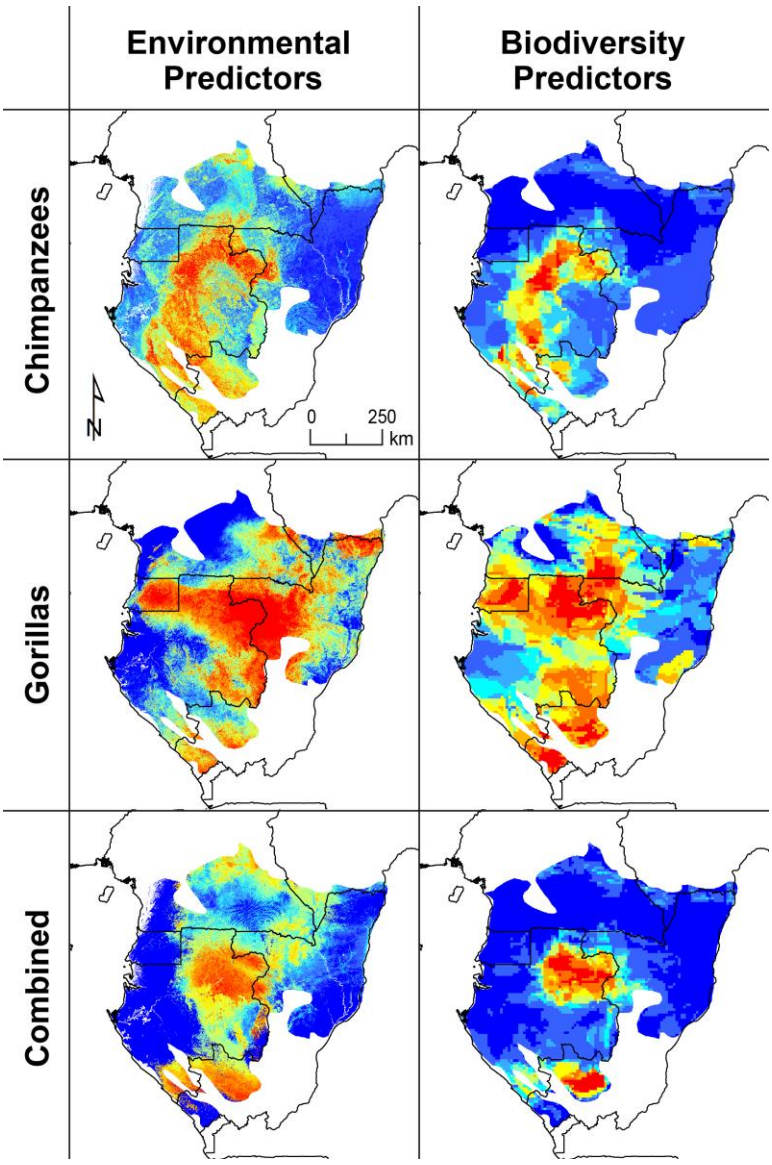

Figure 2. Projected ensemble models for Ebola Virus Disease (EVD) occurrence in Central Chimpanzees, Western Lowland Gorillas, and both combined created using environmental and biodiversity predicting layers. Colors range from blue (lowest estimated probability of EVD occurrence) to red (highest estimated probability of EVD occurrence).

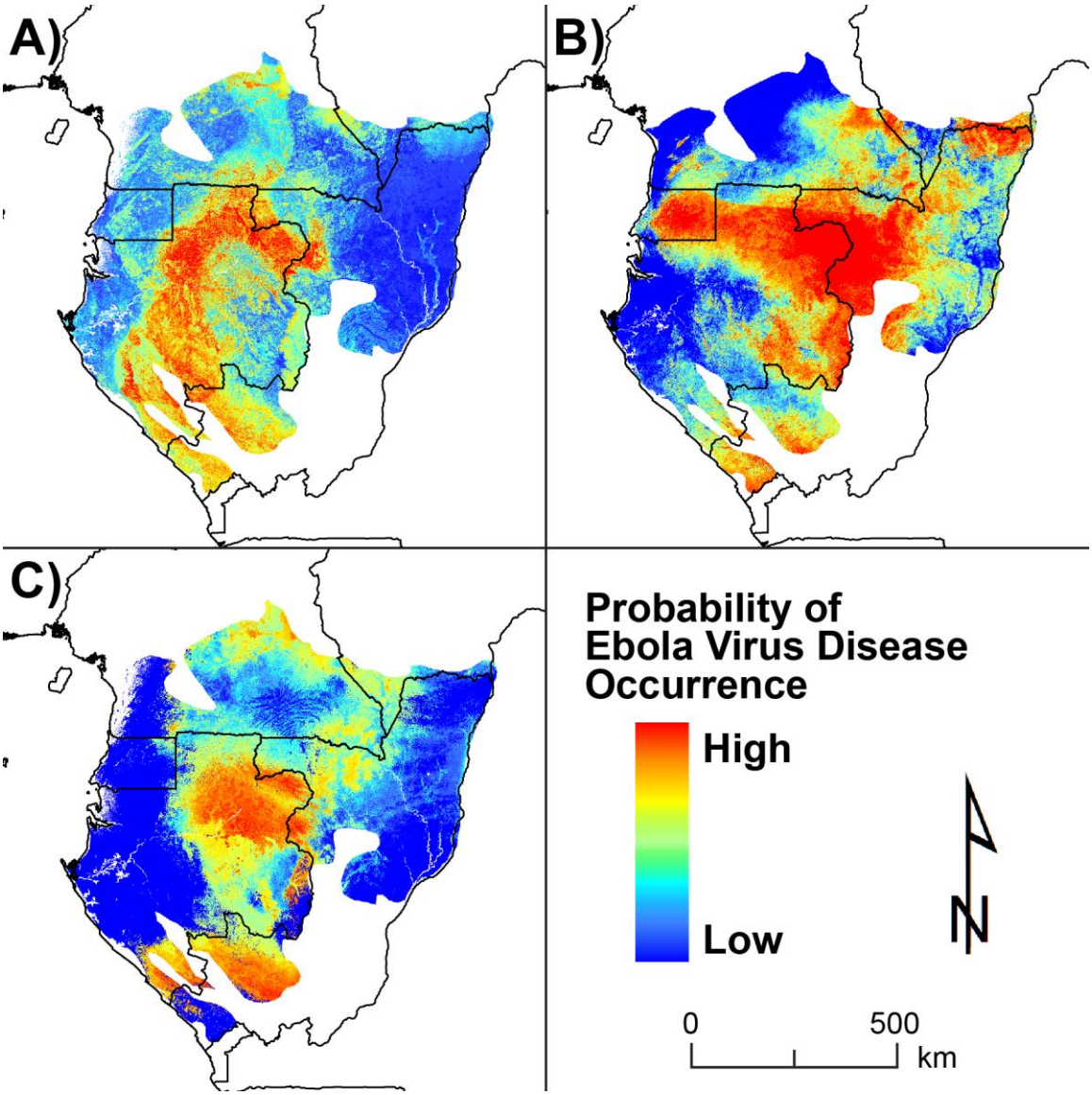

Figure 3. Ensemble distribution models for Ebola Virus Disease (EVD) occurrence in (A) Central Chimpanzees, (B) Western Lowland Gorillas, and (C) combined ape hosts. Areas with low predicted EVD occurrence are blue and areas of highest predicted occurrence are red.

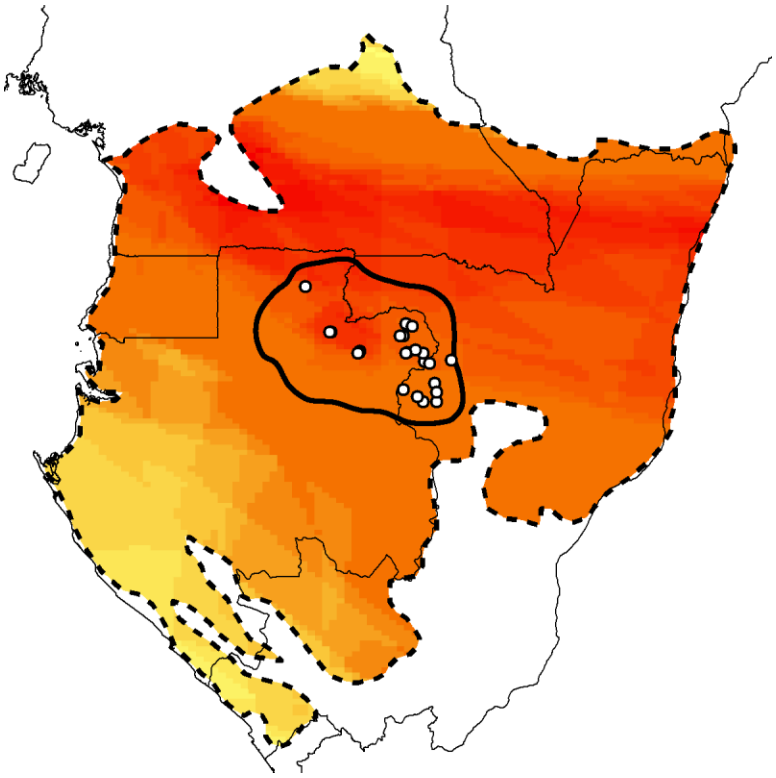

Figure 4. Bat species richness (Yellow, low to Red, high) and Ebola Virus Disease hotspot in chimpanzees and gorillas (outlined in black) with localities of recent outbreaks in these populations (white circles).

Tables

Table 1. Environmental and biodiversity predicting layers used to create ensemble distribution models and to analyze the composition of core areas of Ebola Virus Disease occurrence in chimpanzees and gorillas.

Variable Type	Variable Name	Source
Climatic Factors	Bioclimatic measures of temperature, precipitation, and seasonality	WorldClim; (Hijmans et al., 2005)
Topographic Factors	Elevation	NASA SRTM; (Farr et al., 2007)
	Slope	Derived from above In ArcMap 10 (ESRI, 2013)
Vegetation Indices	Percent Tree Cover	MODIS; (Dimiceli et al., 2011)
	NDMAX – Max. Annual NDVI (Normalized Difference Vegetation index)	MODIS; (Freedman et al., 2010)
	NDMEAN – Mean Annual NDVI	
	NDGR – Max. NDVI of Greening Season	
	NDBR – Max. NDVI of Least Green Season	
	NDGRBR – NDVI Seasonality	
	QMEAN – QSCAT (Surface Moisture Content) Annual Mean	Quick Scatterometer; (Long et al., 2001)
	QSTD – QSCAT Standard Deviation	
Mammal Diversity Indices	Overall Species Richness	(Obtained from or created using Oates et al., 2004; Ceballos et al., 2006; Jenkins et al., 2013b; World Wildlife Foundation, 2016)
	Overall Species Evenness	
	By Order (Carnivora, Certartioda, Chiroptera, Primates, Rodentia)	
	Less than Median Species Richness	
	Threatened Species	

Table 2. Top-contributing variables to ensemble models for Ebola Virus Disease occurrence in Central Chimpanzees, Western Lowland Gorillas, and both combined using environmental and biodiversity predicting layers. Each variable is accompanied by average percent contribution to the ensemble model.

	Ebola Virus Disease Occurrence Models					
	Central Chimpanzees		**Western Lowland Gorilla**		**Combined**	
	Environmental	**Biodiversity**	**Environmental**	**Biodiversity**	**Environmental**	**Biodiversity**
Top Contributing Factors for Distribution Models	Annual Precipitation (bio12) 11.72%	Bat species richness 24.78%	Precipitation of the Driest Quarter (bio17) 13.58%	Bat Species Richness 23.85%	Annual Precipitation (bio12) 11.39%	Bat Species Richness 17.89%
	Percent Tree Cover (MODIS) 10.15%	Overall Mammal Species Richness 16.78%	Mean Temp. of the Driest Quarter (bio09) 12.28%	Overall Mammal Species Richness 17.27%	Maximum NDVI of Least Green Season 10.28%	Overall Mammal Species Richness 13.95%
	Ruggedness 9.61%	Rodent Species Richness 10.74%	Precipitation of the Coldest Quarter (bio19) 8.07%	Even-toed Ungulate species richness 10.04%	Temperature Seasonality (bio04) 9.91%	Rodent Species Richness 13.27%

Chapter 5: TERRA6-AR5: A multi-model ensemble of 30-arcsecond land bioclimatic measures of climate change representative concentration pathways based on the IPCC Fifth Report, with implications for understanding the future of Chytrid in the African tropics

Abstract

Modeling the effects of climate change has become increasingly important for conservation, biodiversity assessments, infectious disease ecology, landscape genetics, public health, and many other fields. These models can have important implications for policy and planning, but rely entirely on the accuracy and strength of the datasets used to create them. Many climatology research groups around the world have released global climate models (GCMs) under guidance from the latest International Panel on Climate Change (IPCC) assessment report. Although each of these models tend to follow the suggestions from the different climatic trends from the IPCC, products from different research groups can deviate from one another due to variation in initial conditions and the mathematical models used to estimate changes over time. When using these projections to model changes in disease distribution over time, it is important to avoid models that fall in the extremes in order to produce in a distribution estimate that aligns well with current standardized climate trends. There are a number of techniques that can be used to reduce a large set of diverse climate models to create a single consensus model that improves the accuracy of distribution model projections. One such method that has been successful involves creating a multi-model ensemble by calculating the

arithmetic mean of a set of existing models for a given study area. This procedure effectively averages the differences between large-scale global models to a baseline that is useful for both global and regional studies.

I used climate change model projections from publicly accessible databases to create TERRA6-AR5 – a high resolution, global multi-model ensemble of bioclimatic measures of climate change. TERRA6-AR5 was created to provide users with a single dataset that summarizes the wide variety of currently available global climate models in order to obtain a more comprehensive estimate of climate change. This dataset consists of bioclimatic layers that encompass different measures of temperature, precipitation, and seasonality for years 2030, 2050, and 2080 across the current four representative concentration pathways from the IPCC fifth Assessment Report at a large geographic extent covering six continents at a spatial resolution of 30-arcseconds (about $1km^2$).

Here, as an example application, I used TERRA-AR5 to create an ensemble distribution model for chytridiomycosis fungal infections caused by *Batrachochytrium dendrobatidis* in amphibians across Africa from known locations of presence and absence across the continent. Present models show that chytridiomycosis occurrence is highest in habitats that are cool and wet with low temperature seasonality and high precipitation. Under the effects of climate change, the projected models show an overall shift of chytridiomycosis presence to higher elevations with sweeping decreases in presence at lower elevations through years 2030, 2050, and 2080. This shift and focus of disease presence coincides with areas

of the most threatened frog and toad species in Africa, and may reduce their chances of survival. Recent studies that have used single-source climate change models show considerable variation between them in terms of the degree of chytridiomycosis distribution expansion or contraction. Using climate change models that lean towards the extremes can result in a range of different projections that could result in different implications for conservation and policy planning.

Introduction

With the recent increased availability of climate change models, a variety of different fields of study have begun to explore how shifts in the Earth's climate may affect species' distributions in the future. Climate change models can be very powerful and useful tools beyond climatology, and have applications in conservation planning on large geographic scales (Razgour, 2015), landscape genetics (Hampe et al., 2005; Sgro et al., 2011), species distribution models (Sesink Clee et al., 2015), and studies of emerging infectious diseases (Ogden et al., 2014). Just as species' distributions may shift overtime to track their ideal climatic niche, pathogens that cause infectious diseases are also expected to undergo similar shifts in their distributions that may alter disease risk for certain hosts or even facilitate the infection of new susceptible hosts in regions where the pathogen was previously absent (Altizer et al., 2013). This is a topic of global importance that can be applied to the spread of diseases in both humans and animals, but climate forecasting relies heavily on the accuracy of underlying modeling assumptions (Peterson et al., 2011).

One potential pitfall in modeling distributions of infectious diseases under climate change lays in the climate models themselves. Individual climate change models are created by research groups from around the world and are typically based on assessment reports from the International Panel on Climate Change (IPCC). After an assessment report is produced, which occurs about twice a decade, climate research groups can use the results as a starting point to develop their own global climate models (GCMs). GCMs are mathematical models that represent the physical changes that may occur on our planet over time under the threat of climate change. These models vary from one another and the results of using different single models to predict distributional changes over time can show significantly different results, therefore it is important to consider not just different climate scenarios, but also the sources of such datasets (Wilby et al., 1999; Hewitson et al., 2006; Van Vliet et al., 2012).

The IPCC fifth Assessment Report (AR5) released in 2014 included four representative concentration pathways (RCPs) to better describe how Earth's climate may change over time than previous socioeconomic-based scenarios (Ipcc - Intergovernmental Panel on Climate Change, 2014). RCPs are greenhouse gas concentration trajectories, numbered 2.6, 4.5, 6.0, and 8.5, which signify the relative radiative forcing values in 2100 from pre-industrial times (Ipcc - Intergovernmental Panel on Climate Change, 2014). Radiative forcing is the difference between the amount of solar energy that is absorbed by the Earth and the amount that is reflected back into space (Hansen et al., 1997), or the net amount of solar energy absorbed. For example, in terms of the RCPs from the IPCC AR5, RCP 2.6 represents

a potential greenhouse gas trajectory that would result from an increase of 2.6 watts per square meter from pre-industrial times by the year 2100. As RCP designations increase in number, they describe situations in which the Earth's atmosphere is absorbing increasingly more solar energy than it is able to reflect back into space. These increases are based on different levels of greenhouse gas concentrations in the atmosphere that perpetuate a 'greenhouse gas effect'. The greenhouse gas effect in terms of global climate works in a similar way to an actual greenhouse. At this scale, the Earth's surface is warmed from solar energy, which is then re-emitted as longer wavelength infrared energy (Oreskes, 2004). In the atmosphere, greenhouse gases absorb this re-emitted energy and reflect it in all directions, including back towards the Earth's surface, which can further contribute to global warming (Mitchell, 1989).

There are many scenarios and models of climate change that are available today, but it is uncertain which most accurately depict the future of Earth's climate. Global climate models use varying initial conditions, parameters, and downscaling techniques that can result is very different climate projections (Wilby et al., 1999). An added layer of uncertainty lies in the RCPs themselves since it is not known which best represents the actual changes in climate that our planet will undergo (Deser et al., 2012). One issue that affects users of climate change model projections is the sheer number of available of options from different sources and the inherent variation between them. There are well-documented methods used to increase confidence when using multiple models in an analysis including (1) min-max, (2) validation techniques, and (3) multi-model ensembles (Fenech et al., 2007). The

min-max approach involves using two models that represent the extremes across a range of different models for a given study region. This technique is useful for observing the ways in which differences among GCMs can result in contrasting outcomes for species distribution predictions. In the validation technique, only models that corroborate with historical climate trends are used, under that assumption that they are likely to be more accurate than models that deviate wildly from observed trends. Lastly, the multi-model ensemble technique involves calculating the arithmetic mean from a large set of models, and is often used in studies for relatively small geographic regions due to complexities of these calculations that occur when using much larger layers (Chaturvedi et al., 2012; Sesink Clee et al., 2015; Wang et al., 2016). These ensembles can be created using different methods such as simple averaging or using Bayesian techniques, both of which show improvements in accuracy over individual models (Giorgi et al., 2000; Murphy et al., 2004).

These various methods that can be used to obtain a more accurate representation of current climate change, but can be difficult for users to produce on their own due to the storage size of the individual files that are used to create ensembles and the computing power required to process them. In an effort to aggregate a variety of different GCMs from publically available databases, I have created TERRA6-AR5: a multi-model ensemble of bioclimatic measures using 20 GCMs for years 2030, 2050, and 2080 for each of the representative concentration pathways put forth by the IPCC fifth Assessment Report (AR5) at a geographic extent covering 6 continents.

Climate change aggregates such as TERRA6-AR5 have a wide range of applications that span a variety of different fields of study. One of the most evident uses involves incorporation into distribution or ecological niche models. In this study, I modeled the presence of chytridiomycosis infections caused by the fungus *Batrachochytrium dendrobatidis* in amphibians across Africa using TERRA6-AR5 implemented in Biomod2 (Thuiller et al., 2013). *B. dendrobatidis* is a water-borne pathogen that disperses via zoospores that use flagella to move through aquatic environments and settle in the epidermis of amphibian hosts (Longcore et al., 1999). Zoospores then develop into sporangia, producing additional zoospores that can disperse through water or reinfect the same host individual (Berger et al., 2005). Symptoms of chytridiomycosis infections in amphibians include epidermal lesions and the thickening of outer epidermal layers, leading to increased sloughing of skin, lethargy, abnormal posture, reddening of ventral skin, spasms/extensions of hind limbs, and failure to seek shelter and flee when provoked (Berger et al., 1998; Carey et al., 2006). The thickening of skin and increased sloughing appear to be the major causes of mortality as they severely limit individuals' ability to release toxins, absorb nutrients, and respire (Pessier, 2002). Chytridiomycosis infections have been linked to amphibian declines and extinctions across the world in tropical and temperate regions including South America, Central and Southern Africa, Southeast Asia, Australia, and parts of North America and Europe, but there have been few studies identifying the effects of climate change on its distribution (Berger et al., 1998; Collins et al., 2003; Bosch et al., 2007; Reeder et al., 2011b; Xie et al., 2016).

Materials and Methods

Climate Change, Agriculture, and Food Security (CCAFS) maintains a current database of map layers from a variety of GCMs that are publically accessible for users to download and use for their own research (Ramirez et al., 2008). All available models with global coverage at 30-arcsecond resolution (about 1km^2) at the time of its creation were downloaded to processing. Suitable models that were only available as raw temperature and precipitation variables were converted to bioclimatic measures following the methods from Ramirez-Villegas et al. (2009). Models at coarser spatial resolutions or only regional extents and models that were not available for all RCPs were omitted from the final aggregate, since there is little benefit in artificially increasing the spatial resolution of a climate projection without underlying detailed climate data. Models were also only selected if there were available projections for years 2030, 2050, and 2080 so that no ensemble from a single period would include different contributing models to another period. The arithmetic mean of the combined set of 20 GCMs (Table 1) was calculated using the ArcMap 10 Model Builder and Spatial Analyst Toolbox (ESRI, 2013).

Coordinates of chytridiomycosis occurrence from sampling of wild amphibians across Africa were collected from individual publications (Hopkins et al., 2003; Goldberg et al., 2007; Kielgast et al., 2010; Bell et al., 2011; Conradie et al., 2011; Daversa et al., 2011; Reeder et al., 2011a; Weldon et al., 2011; Balaz et al., 2012; Doherty-Bone et al., 2013; Gower et al., 2013; Tarrant et al., 2013; Bletz et al., 2015) and from the Bd-Maps database (Olson et al., 2013) which assembles reports of chytridiomycosis or *Batrachochytrium dendrobatidis* infected amphibians from

current publications. Coordinates of both the *Bd*GPL (global) and *Bd*CAPE (Southern African) lineages (Farrer et al., 2011; Bletz et al., 2015) were collected for this study to cover the major strains present in Africa. Coordinates with *B. dendrobatidis* infected amphibians were categorized as presence localities and coordinates with lack of *B. dendrobatidis* infections were categorized as absence localities. Environmental predicting factors used to create these models included measures of temperature, precipitation, and seasonality (Hijmans et al., 2005).

Biomod2 can be used to apply inputted presence/absence dataset and environmental predictors to up to 11 different distribution modeling techniques to the to create and project models of estimated presence as a function of underlying environmental measures. Each model is tested for its individual performance and used to create a weighted ensemble model that can be projected across the study region. Models in this study were created for each lineage of *B. dendrobatidis* using 10 replicates for each of the 11 different models for a combined total of 110 different models used to create the final ensemble projection. Final ensembles were weighted by their true test statistic (TSS) and receiver operating characteristic (ROC), while poorly performing models with a performance threshold of 0 for TSS (which ranges from -1 to +1) or 0.6 for ROC (which ranges from 0.5 to 1). These ensemble models were projected under TERRA6-AR5 for RCPs 2.6, 4.5, 6.0, and 8.5 for years 2030, 2050, and 2080 at a 30-arcsecond (about $1km^2$) spatial resolution for the continent of Africa. A conservative presence cutoff of 0.8 (on a scale from 0, absence, to 1, highest estimate presence) was applied to each projection. The resulting binary maps were used to calculate the total estimated change in disease

distributions over time. Lastly, projections were assessed across much of the Cameroon Volcanic Line region since it appeared to be a location of rapid shifting in pathogen occurrence. Across this sub-region, layers of amphibian species richness by order (Anura, Apoda, and Caudata) and threat of extinction (Jenkins et al., 2013b) were used to assess whether any specific type of amphibian appeared to be under the most threat from shifting ranges of *B. dendrobatidis* occurrence.

Results

TERRA6-AR5 consists of 19 bioclimatic layers for RCP 2.6, 4.5, 6.0, and 8.5 for years 2030, 2050, and 2080. Each layer has a spatial resolution of 30 arc-seconds (about $1km^2$) covering land at a global extent. This dataset was created with large continental and global studies in mind and is available as complete layers to eliminate the need for users to stitch small tiles. Due to the availability of data from the CCAFS climate database at the time of its creation, TERRA6-AR5 includes bioclimatic layers for six of the seven continents. TERRA6-AR5 aggregates will be available for download in ARC GRID format in decimal degrees and datum WGS84. All layers have been tested for alignment and are prepared for use in external mapping and analysis programs. TERRA6-AR5 will be updated over time, which will include breaking layers into a tiled grid for regional studies, releasing addition file formats such as ASCII rasters, inclusion of more GCMs as new products are released, and additional coarser spatial resolutions.

Present models of *B. dendrobatidis* across Africa created using Biomod2 show that its distribution is driven primarily by mean temperature of the wettest quarter,

annual precipitation, mean diurnal range, and temperature seasonality. Collectively, the highest estimated presence for chytridiomycosis is found in areas that experience cool wet seasons, high levels of precipitation, and minimal temperature fluctuations between wet and dry seasons. All 11 models performed exceptionally well (ROC > 0.8) and none were removed due to poor performance. Generalized boosting models, classification tree analysis, and generalized additive models performed best overall with performance scores greater than 0.9 ROC. Present ensemble models were projected under climate change scenarios using all RCPs from TERRA6-AR5 for years 2030, 2050, and 2080. These projections show distributions of chytridiomycosis presence condensing and shifting to higher elevations over time, with a rapid decrease of overall presence at low elevations (Figures 1 and 2). The elevational shift is most evident when focusing on areas that exhibit pronounced elevational changes, such as over the Cameroon Volcanic Line (Figure 2). The highest elevations found in this region are upwards of 4000 meters, and the highest probability of *B. dendrobatidis* occurrence is projected to shift and condense between 1500 and 3000 meters above sea level in coming decades. This elevational range coincides with the distributions of several threatened amphibians, particularly in frogs and toads (Order Anura) (Jenkins et al., 2013b). Areas with the most threatened amphibian species shift from an average of 23.2% overlap with chytridiomycosis presence estimates under current conditions to over 70% by year 2050 under all future RCP projections.

Discussion

Batrachochytrium dendrobatidis infections under climate change

Chytridiomycosis is a fungal infection caused by *Batrachochytrium dendrobatidis* that has been detected in amphibians around the world, causing mortality (Lötters et al., 2009) that has led to local extinctions in the recent past (Pounds et al., 2006; Wake et al., 2008). When the fungus infects an amphibian, motile zoospores that traverse through aquatic environments settle in the epidermis of amphibians where they can develop in sporangia that release more zoospores into the water that can infect more individuals (Berger et al., 2005). Progressive forms of the disease appear after about 15 days, and are characterized by lethargy, increased sloughing of skin, and sporadic movements in limbs (Carey et al., 2006). The combination of limited respiration through their skin along with reduced movement can lead to mortality in only a few days. Its rapid spread in amphibians around the world is a major concern for conservation initiatives to prevent widespread declines of amphibian populations that could have downstream consequences with increases in insect populations and long-term changes in the composition of other consumers and algal communities (Whiles et al., 2006). Currently, it appears that parts of tropical Africa are experiencing little decline in amphibian populations due to chytridiomycosis infections compared to amphibians in temperate environments (Penner et al., 2013), but changing climate may play a role in its spread over time and introduce it to more vulnerable populations (Bosch et al., 2007).

In this study, I used TERRA6-AR5 climate projections to model the distribution of *B. dendrobatidis* infections in amphibians across Africa using ensemble niche modeling techniques. Under present conditions, *B. dendrobatidis* infections are found in regions that have cool temperatures, high precipitation, low temperature seasonality, and small diurnal ranges. High rainfall and relatively even temperatures across the year can foster the persistence of water-borne pathogens and may help explain the lack of chytridiomycosis infections in areas with low annual precipitation or more distinct wet and dry seasons (Sharma et al., 2003). This model agrees with general trends shown in some previous studies that modeled the effects of climate change on *B. dendrobatidis* over large scales and showed overall decrease in presence across Africa (Rödder et al., 2010), but there are still disagreements in the expected geographical patterns of chytridiomycosis distributions over time (Pounds et al., 2006; Alford et al., 2007; Lips et al., 2008; Xie et al., 2016).

These future models of chytridiomycosis infections under AR5 representative concentration pathways show an overall decrease in presence over time at low elevations by as much as 85%, coupled with an increase of presence in higher altitudes by up to 42%. A recent study by Xie et al. (2016) projected chytridiomycosis distributions under climate change scenarios for the year 2100 using the random forests model and included global and regional models that reported an expansion of chytridiomycosis presence over time. Their models are difficult to assess for the tropics, where by around 2080, climate is predicted to shift beyond existing ranges, vastly increasing uncertainty in distribution model

projections due to their unknown effects on the physiology of animals in natural settings (Mora et al., 2013; Corlett, 2015). The models presented in my study include the more comprehensive TERRA6-AR5 climate projections and also forecasts changes in increments up to 2080. The combined effect of this refined modeling procedure shows pronounced decreases in *B. dendrobatidis* occurrence at low elevations, even by year 2030, and increase of the fungus at higher elevations (1500-3000 meters above sea level). The marked decrease of occurrence at low elevations observed in these models results in a net loss of estimated presence and geographic coverage. Granted, worst-case RCPs, such as 6.0 and 8.5, show more *B. dendrobatidis* presence around this altitude, but still show greater declines and loss of presence from the current range of chytridiomycosis infections at lower elevations. Biomod2 also showed that random forests (ROC = 0.845) was outperformed by generalized boosting model, generalized additive model, and classification tree analysis (ROC = 0.912, 0.904, 0.967 respectively) for predicting chytridiomycosis presence across Africa. Differences in projected distributions between current models from this and other studies may be due to the modeling techniques that were used, time periods used for future projections, or the climate change models themselves.

The decline of chytridiomycosis occurrence becomes more apparent when focusing on the steep elevational gradient of the Cameroon Volcanic Line. Coastal regions where rainfall is among the highest in the world, exceeding 5 meters annually, appear to be a haven for high diversity in amphibians including frogs, toads, salamanders, and caecilians (Poynton, 1999; Penner et al., 2011). Ensemble models

predict that in this region, chytridiomycosis infections will undergo an elevational shift from lowlands to around 2000 meters above sea level, which coincides with a hotspot for endangered and threatened amphibian species (Jenkins et al., 2013a). It is estimated that this elevational range along the Cameroon Volcanic Line encompasses the core habitat for over 15 threatened frog species including *Leptodactylodon bueanus* (vulnerable), *Astylosternus perreti* (endangered), and *Astylosternus rheophilus* (vulnerable) (IUCN, 2016).

It is important to consider for this region, and similar ones around the continent, that threat of amphibian declines due to disease are compounded with the effects of climate change on the host taxa themselves due to their critical thermal tolerances (Hof et al., 2011). When modeling the presence of a disease infecting host taxa under the effects of climate change, the livelihood of the host taxa may also be at risk. These models showing *B. dendrobatidis* infections shifting to higher elevations do not necessarily mean that all amphibians at lower elevations will experience declines. In the past, amphibians have been able to track changes in climate, causing shifts in suitable habitats, but this may prove more difficult with the predicted speed of change shown in more severe current models of climate change (Carey et al., 2003). Amphibian distributions on large scales are affected by different agents including climate change, infectious disease, and anthropogenic land-use change, but disease is widely regarded as their most immediate threat of declines (Lötters et al., 2009; Hof et al., 2011).

Climate change datasets and their applications

Multi-model ensembles have been shown to improve overall predictive power and reliability when compared to single models, and have applications in climatology (Gneiting et al., 2005), agriculture (Palmer et al., 2004), disease dynamics modeling (Morse et al., 2005; Thomson et al., 2006), and many more fields. This general technique involves taking different models of climate change and combining them to create a single model that averages the differences between them (Tebaldi et al., 2007). TERRA6-AR5 encompasses a large variety of different models that were created using distinct initial conditions and modeling methods. Errors may exist in any of these single models, and combining them to determine a consensus results in a stronger overall model that balances their individual differences. This climate change ensemble will be especially useful for users seeking a single product that averages the inherent variation between GCMs without having to summarize the data themselves. As with any climate change dataset, one must consider that each RCP is only one potential track that climate change may take (Hansen et al., 1997). These RCPs cover a wide range of climate change trajectories that include minor to major shifts, so it is important , when possible, to consider all levels of severity in analyses. When using layers such as these to project a distribution over time, it is also essential to consider the possibility that a species' niche requirements may change.

Distribution models of infectious diseases are one among many potential applications of the TERRA6-AR5 dataset. In studies of population genetic structure within taxa, climate models can be used to detect potential geographic shifts, which

may contribute to speciation events in the near future (Davis et al., 2001b; Ayre et al., 2004; Carstens et al., 2007). Models of climate change can also be used in applications more closely involving humans, such as studies of agriculture and water availability in rural communities that rely on their crops to survive (Howden et al., 2007; Schmidhuber et al., 2007; Hanjra et al., 2010; Schlenker et al., 2010). Furthermore, climate change models can be used to identify regions and target key areas to focus adaptation and mitigation efforts to combat the consequences of global warming (Smit et al., 2000; Adger et al., 2003; Intergovernmental Panel on Climate Change, 2014).

When using models of climate change in analyses, users should consider a baseline uncertainty with any of these predictions. Although current climate models appear to be robust and statistically strong, there is uncertainty about which of these models will be closest to reality (Stainforth et al., 2005). By incorporating all current RCPs, studies can examine the average of a range of potential effects of climate change over time and use these results to predict large-scale trends that may occur. TERRA6-AR5 is a constantly evolving multi-model ensemble of climate change scenarios that covers 6 continents with 30-arcsecond spatial resolution. Applicable new climate models will be incorporated over time to stay current with the release of new available climate change research. This dataset includes a more diverse set of individual GCMs at a higher spatial resolution and larger geographic extent than previous multi-model ensembles of climate change, which tended to focus on smaller, more regional scales. TERRA6-AR5 will remain a free product

with creative commons use, and users are encouraged to create derivative datasets for their own research and to share with other researchers.

Figures

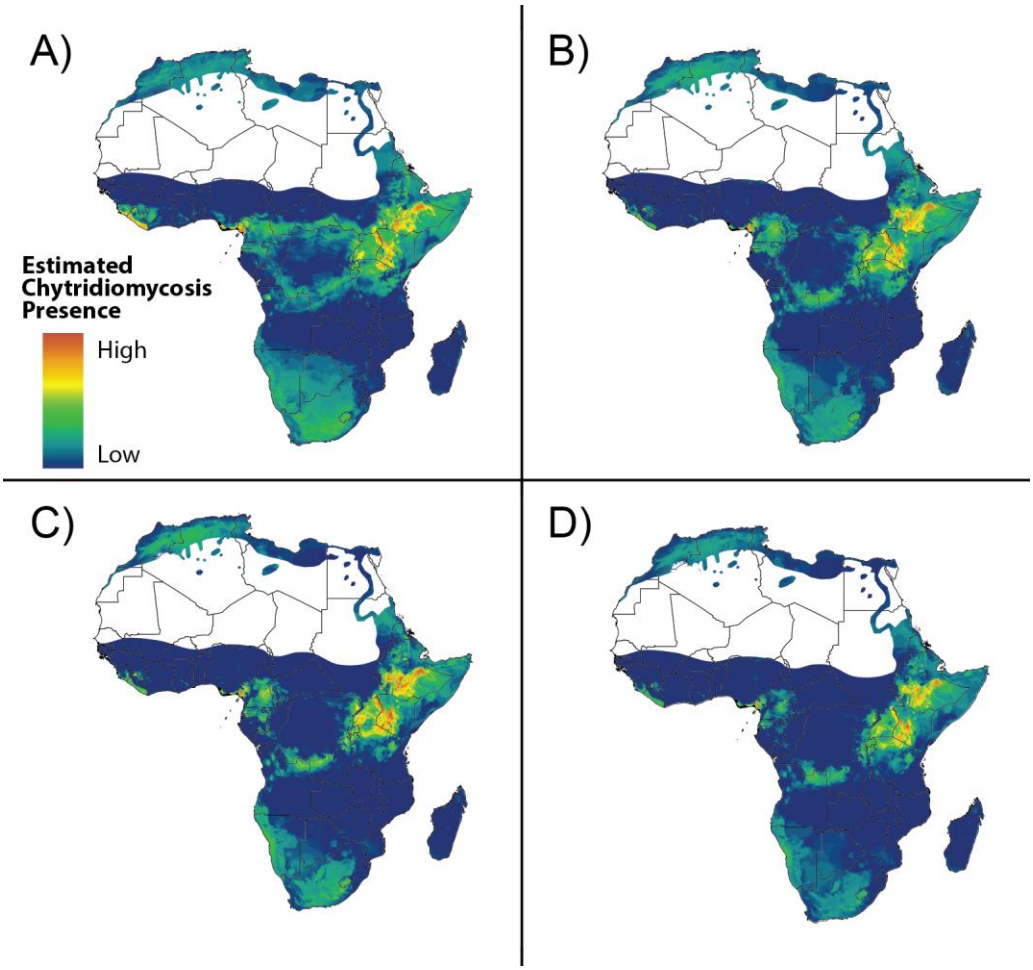

Figure 1. Estimated presence of chytridiomycosis under RCP4.5 from the IPCC fifth Assessment Report across Africa. An ensemble distribution model for chytridiomycosis was projected under (A) present conditions, and future conditions for years (B) 2030, (C) 2050, and (D) 2080. Warmer colors indicate high estimated probability of presence and cooler colors represent low estimated probability of presence.

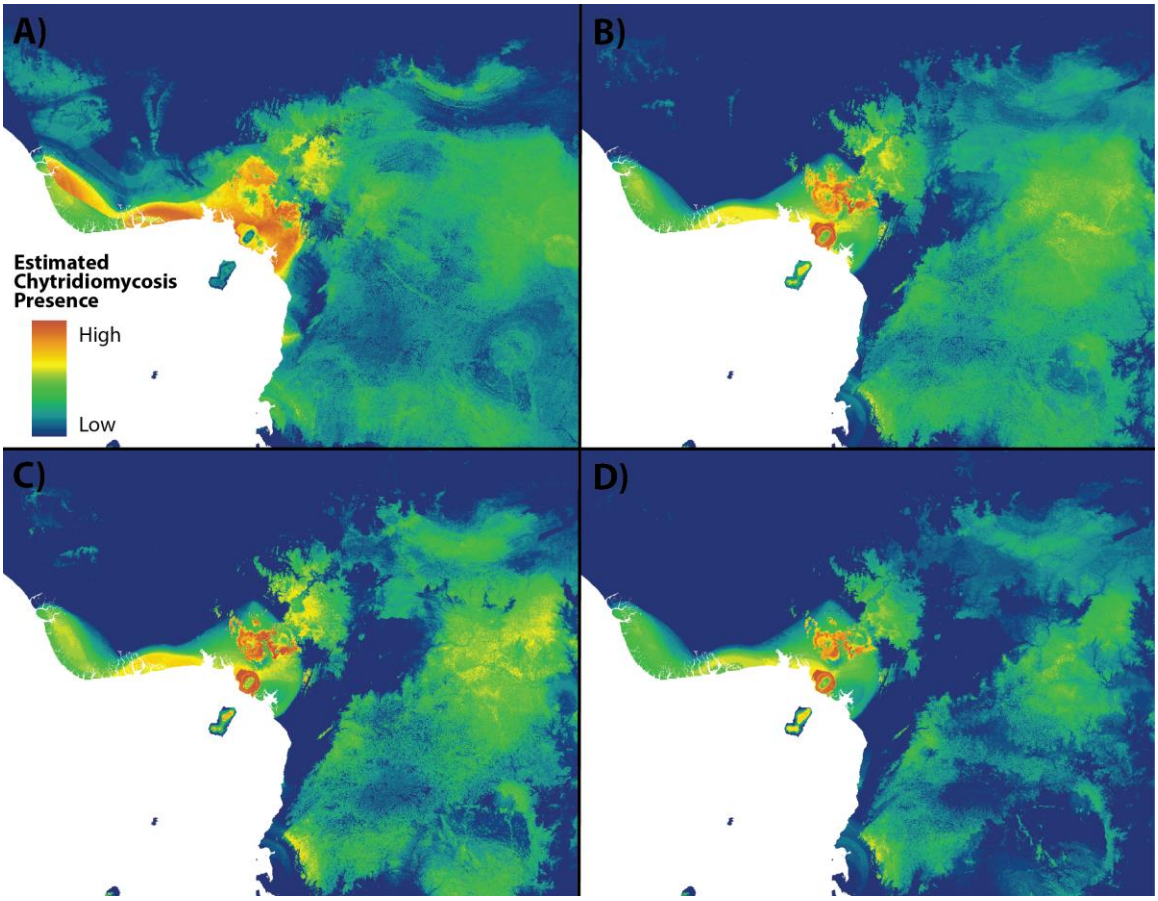

Figure 2. Continent-wide model of Chytridiomycosis presence the under RCP4.5 from the IPCC Fifth Assessment Report with a focus on the Cameroon Volcanic Line, including the Cameroon Highlands, Mount Cameroon and Bioko Island, Equatorial Guinea. Maps represent estimated disease occurrence under (A) present conditions, and future conditions for years (B) 2030, (C) 2050, and (D) 2080. Warmer colors indicate high estimated probability of presence and cooler colors represent low estimated probability of presence.

Tables

Table 1. GCMs included in final bioclimatic aggregates

Global Climate Models Included in Aggregate	Group Name
BCC_CSM1_1_m	Beijing Climate Center, China
CCSM4	National Center for Atmospheric Science, CO, USA
CESM1_CAM5	Community Earth System Model, CO, USA
CNRM-CM5	National Centre for Meteorological Research
CSIRO MK3	Commonwealth Scientific and Industrial Research Organisation, Australia
FIO_ESM	First Institute of Oceanography, China
GFDL_CM3	Global Land Cover Facility, MD, USA
GFDL_ESM2G	
GFDL_ESM2M	
GISS_E2_H	NASA Goddard Institute for Space Studies, NY, USA
GISS_E2_R	
INM-CM4	Institute of Numerical Mathematics
IPSL_CM5A_LR	Institut Pierre Simon Laplace, France
IPSL_CM5A_MR	
MIROC_ESM_CHEM	Model for Interdisciplinary Research On Climate, Japan
MIROC_ESM	
MIROC5	
MRI_CGCM3	Meteorological Research Institute, Japan
NCC_NORESM1_M	National Climate Centre of China Meteorological Administration, China
NIMR_HSDGEM2_AO	National Institute of Meteorological Research, Korea

Chapter 6: Biomod2EZ – An R script suite for visualizing projected niche model ensembles and reporting statistical results

Introduction

Ecological niche modeling is a technique that involves predicting the distribution of a species as a function of underlying ecological patterns across a study region and has become increasingly useful in studies involving biodiversity, conservation, and disease occurrence. Use of these models in research projects has increased dramatically in recent years with the ever-increasing availability of environmental map layers created from remotely sensed data. One issue for ecologists using niche models in their research is the wide variety of different mathematical models that can be used to create these projections. While one model may perform well for a given dataset, it may perform poorly with a different dataset. The variety of available models alone can make research establishment a daunting task.

Biomod2 (Thuiller et al., 2013) is an R package that can currently be used to run up to 11 different niche models using a single input dataset, calculate each model's individual performance strength, and create an ensemble model weighted by these individual model performance scores. Models that perform poorly (determined by a user-defined cutoff value) can be completely excluded from the ensemble. This modeling technique has revolutionized the way that users can create ecological niche model projections without the need for in-depth knowledge of which single model is best suited for a given dataset. *Biomod2* reduces the risk of choosing an ill-

fitting model by combining several different models into a final ensemble projection. Unfortunately for users that are unfamiliar with using R (R Development Core Team, 2016), *Biomod2* lacks a simple visualization of results that would be useful in post-analysis.

Functional Description and Advantages

This R script suite aims to simplify the use of *Biomod2* (Thuiller et al., 2013) for ecological niche modeling and generate reports of model results. Many ecologists that are familiar with using Graphical User Interfaces (such as Maxent: Phillips et al., 2006) are reluctant to create ecological niche models using the more complicated coding environment of the R framework (R Development Core Team, 2016). This suite includes fully annotated scripts for users to input their data (i.e., species presence/absence, environmental layers, etc.) and create ensemble niche models, which are exported directly into the working directory as an ASCII raster for use in external mapping programs.

Biomod2EZ includes four scripts along with an *rmarkdown* file for report generation and a .jar file of the current version of Maxent (Phillips et al., 2006). The first script assists users in importing their presence/absence data, environmental predicting layers, and optional layers for projection (eg. for projecting models to different geographic locations or under different climate scenarios). The second script is used to prepare the modeling environment and ensure that all associated packages are properly installed. The third script can be used to optionally remove duplicate presence and absence points that share the same coordinate. A final script contains

all necessary steps to create niche models and ensembles along with modeling parameters that should be modified by users to best suit their datasets.

Most significantly, this R script suite improves the functionality of the *Biomod2* package by automatically generating reports that include output maps, performance scores for individual models, ensemble projections, decision trees for species presence/absence based on the provided environmental layers, environmental variable contribution to each individual model, indication of any failed models, and descriptions of individual models and test statistics used to determine model performance.

Software Availability

This R scripts suite and included sample dataset/tutorial is available for download at: http://www.pages.drexel.edu/~mkg62/biomod2ez

Acknowledgements

Biomod2 is a product of Thuiller et al. (2013) and is implemented unaltered in this script suite to simplify use and generate a visual report of results.

Biomod2EZ Manual and Tutorial

December 21, 2016

Type - Script Suite

Title - Report Generation for Species Distribution Modeling with Biomod2

Version - 1.0

Date - 2016-12-21

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Description - Set of scripts to simplify the use of Biomod2 for species distribution modeling and ensemble creation with addition summary report generation features that are absent in the standard Biomod2 release.

Depends - R (>=3.2.1), biomod2, stats, utils, sp, raster, parallel, reshape, ggplot2, rgdal, base, rpart, rpart.plot, rattle, readr, pander, stringr, rmarkdown

I. INTRODUCTION

The goal of this script suite is to simplify the use of *Biomod2* (Thuiller, 2016) for creating ecological niche models and ensembles for a given study taxa. *Biomod2* can be used to create and project distribution models, but it falls short in its lack of report generation to concisely display results in an easily digestible format. If lengthy models are run without saving the results from the R console, users can lose information such as environmental predictor contributions and model performance scores. Additionally, saving results manually can become tedious when running multiple models in series. *Biomod2EZ* includes scripts that simplify and thoroughly explain all steps in the modeling process for new users, while incorporating a new report generation feature that provides users with an exported file that summarizes model results, ensemble projections, model performance scores, and environmental predictor contributions.

Biomod2EZ is distributed as a compressed file that should be extracted into a user's working directory. *Biomod2* is a product of Thuiller et al. (2016) and is implemented unaltered in this script suite to simplify use and generate a visual report of results. This README should be read carefully before using the script suite for model production.

II. SCRIPT FUNCTIONS

1_DataInput_ModelInitiation.R

Presence/Absence Data

The table of presence and absence coordinates should have the following columns: (1) X coordinate column consisting of decimal degree longitude designation; (2) Y coordinate column consisting of decimal degree latitude designation; (3) species column used to identify points as presence ("1") or absence ("0") of your study taxa. This file must include a header row with column labels. For an example file, see: *Biomod2_EZ_SampleData/presabs.csv*

Environmental Variables

Map layers for the target study region should have identical extents and spatial resolution. *Biomod2* can read different raster formats, but using ASCII grid (**.asc*) formatted files is suggested for universal compatibility between different packages/programs. It is good practice to keep all layers organized in the same folder to simplify the input of filepaths.

If you want to project your model to different layers or under climate change scenarios, you must uncomment the bottom section that contains a template raster stack, and enter filepaths to these projection layers with the same names as present layers used to train the model.

DedupingPresAbs.R

This supplemental script can be used to remove duplicate presence and absence points (independently) from a presence/absence table file. This is a useful step to clean your dataset and can help models run quickly while reducing biased sampling.

InstallingPackages.R

This script can be called directly from the first script (1_*DataInput_ModelInitiation.R*). It contains loops that check for, install, and load a variety of packages that are used by this script suite to accomplish data extraction, model building, and report generation.

ParametersAndReportGeneration.R

This script can be called directly from the first script (1_*DataInput_ModelInitiation.R*), but it is advised that you instead run it in blocks while becoming familiar with this script suite. This will help you pinpoint issues with your datasets, should any errors occur. For an exhaustive description, see

the following section and detailed script annotation.

Biomod2_Report.Rmd

This *rmarkdown* file is used to combine exported model results/maps/tables into a rendered report HTML file.

Maxent.jar

This java executable is used by Biomod2 to run Maxent models (Phillips et al., 2006).

III. MODEL PREPARATION AND GENERATION

Loading Data

BIOMOD_FormattingData()

Formatting user-inputting data for use by *Biomod2*. Actions include: assigning presence and absence designation to *resp.var*, species coordinates to *resp.xy*, species name to *resp.name*, and raster stack of environmental layers to *expl.var*.

BIOMOD_ModelingOptions()

Setting parameters for individual models. This block is mostly commented out by default, but it is suggested that you research each model, and determine parameters that will best suit your dataset.

Running Models

BIOMOD_Modeling()

Setting parameters for individual models. Options include: deciding which of the available models you want to run (*GLM* , *GAM* , *GBM* , *SRE*, *CTA*, *ANN* , *FDA*, *MARS*, *RF*, *Maxent.Phillips*, *Maxent.Tsuruoka*), number of replicates for each model (*NbRunEval*), splitting your dataset for training/testing (*DataSplit*), and evaluation metrics for models (*models.eval.meth* = *TSS*, *ROC*, *KAPPA*) among other parameters that are detailed in script annotations.

Creating Ensemble

BIOMOD_EnsembleModeling()

Setting parameters for ensemble forecasting. Options include: choosing which models to include in the ensemble (*chosen.models*), evaluation metrics (*eval.metrics*), and a set of options that involve the weighting of contributing models.

BIOMOD_Projection()

Setting parameters for projecting individual models. Options include: setting a new raster stack for projecting under climate scenarios or to a different area (*new.env*), selecting which models to project (*selected.models*), and output projection format (*output.format*).

BIOMOD_EnsembleForecasting()

Projecting ensemble model defined by BIOMOD_EnsembleModeling(). Additionally, the projected ensemble map is converted it to an ASCII grid raster and exported to the working directory for use in external mapping software.

Variable Decision Tree

This section uses the user-inputted presence/absence data to create a simple decision tree based on underlying environmental variables. This is performed by converting coordinates to the *SpatialPoints* format to ease the process of extracting environmental data at each point. These extracted variables are saved as a table to the working directory (*xy_value_extract.csv*). Finally, the *rpart* package is used to build a tree which is plotted using *rpart.plot*::*fancyRpartPlot*() and saved to the /Plots folder in the working directory as a PNG.

Generating Report

This block is used to export data structures and images for generating the final report using *rmarkdown*. An included function loops through a list of all model names and checks to see if each model was run successfully. If a model worked, the map is exported to the /Plots folder in the working directory as a PNG. If a model failed, it copies a blank placeholder image for display in the report.

Portions of the BiomodModelOut and BiomodModelEval objects are saved as R objects (RDS). These include: evaluation methods for all models and iterations that were run, variable importance for each iteration, and evaluation of the ensemble. Finally, *rmarkdown* is used to call and render *Biomod2_Report.rmd* to an HTML file that organizes all results and analyses. Note that there is also commented out code that can be used to instead export reports as PDFs (this requires a properly initialized LaTeX installation).

IV. REPORT COMPONENTS

Ensemble Projection

Displays map of the resulting ensemble model projection.

Testing Model Performance

Receiver Operating Characteristic (ROC, Hanley et al., 1982)

The area under the *ROC* curve is commonly used as a standalone measure of model performance. Values range from 0.5 to 1.0 where low values reflect a model that is no better than a random association between species presence/absence and underlying environmental variables and high values close to 1.0 reflect a very strong signal of association.

True Skill Statistic (Hassen-Kruipers discriminant) (TSS, Monserud et al., 1992)

TSS compares the number of correct forecasts to a hypothetical set of perfect forecasts. Values range from -1 to +1, where values less than zero indicate that the model performs no better than random and values close to 1 perform very well. *TSS* is similar to *KAPPA*, but it is not affected by prevalence or by the size of the validation set.

Cohen's KAPPA (Allouche et al., 2006)

This metric uses the accuracy expected by chance to correct overall accuracy of a model. Values range from -1 to +1, where values less than zero indicate that the model performs no better than random and values close to 1 perform very well. It should be noted that *KAPPA* is criticized for being dependent on prevalence, introducing bias to estimates of accuracy.

Individual Model Types

Generalized Linear Model (GLM)

Linear regression model that allows for non-linearity and is based on an assumed relationship between the response variable and predictor variables (Nelder et al., 1989).

Generalized Boosting Model (GBM)

A powerful machine-learning algorithm that can be used to fit regressions,

perform classifications, and determine ranking. It applies boosting methods to regression trees by creating simple trees where each tree is based on the prediction residuals of the previous tree and each node is set on a binary decision. Each subsequent tree is used to find a new partition in the dataset that can further reduce error (Ridgeway, 2007).

Generalized Additive Model (GAM)

Combines aspects of both additive models and generalized linear models. They function like a *GLM* in that they can have different error structures and link functions, but instead of having an explicit functional form, the relationship uses non-parametric smoothers to describe the relationship. They are useful for distributions that have complex shapes (Hastie et al., 1990; Hastie, 2013).

Classification Tree Analysis (CTA)

A type of machine learning algorithm used for classifying remotely sensed data in support of land cover mapping and analysis. Classification trees structurally determine binary decisions to estimate the dependent variable (Therneau et al., 2010).

Artificial Neural Network (ANN)

A computational model based on the way that biological neural networks function. This type of model changes while information is fed through it and, in a sense, learns how to improve the model over subsequent iterations (Ripley et al., 2011).

Surface Range Envelope (SRE)

Same as *BIOCLIM* . Determines habitat suitability at each grid cell by comparing values of environmental variables there to a percentile distribution of variables at locations of known presence. The closer that habitats across the study region are to known suitable habitats (at locations of species presence), the more suitable the location is deemed (Busby, 1991).

Flexible Discriminant Analysis (FDA)

Used to predict a categorical dependent variable (i.e. presence or absence) using one or more predictor variables. It is also known as 'pattern

recognition', 'supervised learning', and 'supervised classification'. This differs from a cluster analysis, which is unsupervised. Objects with known groups are used to then determine the category that ungrouped objects fall in. This is done by identifying relationships among groups' covariance matrices to be able to discriminate between different groups. It is important to note that with *N* number of groups, the model requires *N* – 1 number of predictor variables (Febrero-Bande et al., 2012).

Multiple Adaptive Regression Splines (MARS)

Non-parametric regression technique that automatically models nonlinearities and interactions between variables. Starting with the mean of the response variables, the model finds basis functions that result in the smallest residual error. Each consecutive basis function consists of one term that is already in the model multiplied by a new hinge function (consisting of a variable and a knot). The *MARS* model, when creating new basis functions, must scour over all possible combinations of existing terms and all variables (Milborrow, 2013).

Random Forests (RF)

Non-parametric regression technique. Response is tested against predictor variables, and the model tries to split response variables into 2 groups that have the smallest amount of variation (presence vs. absence) in each part (this continues until it builds a full decision tree). Variables can show up in multiple locations in the tree. Pruning trees defines where to stop tree building (after how many levels). Each run randomizes presence/absence points used and environmental predictors used without using all of them at once. This allows the model to determine which variables make the model performance drop when removed (highly important variables) across many trees. With RF, you do not necessarily need to reduce the predictor set because it will only use the best variables for the final model (Liaw et al., 2002).

Maxent (MAXENT.Phillips)

Uses environmental data at known presence localities and a set of background points (or pseudoabsences) in a machine learning methodology using the principle of maximum entropy to model species distributions. This process chooses models with uniform/spread-out distributions while considering the study region as a density estimation of presence (Phillips et al., 2006).

Maxent (MAXENT.Tsuruoka)

Unlike the Phillips version that runs using java, the R package to implement a maximum entropy approach in species distribution modeling focuses on minimizing memory consumption for large datasets and is based on an efficient C++ implementation (Tsuruoka, 2006).

Ensemble Evaluation

Table showing the results of the model evaluation statistics from the list that is defined by *models.eval.meth*.

Presence/Absence Decision Tree

Environmental variables are extracted at each presence/absence point and are saved to a table in the working directory (*xy_value_extract.csv*). They are then used to create a *SpatialPoints* object to create a simple classification tree using the rpart package (Therneau et al., 2010).

Variable Importance

Relative importance of each variable calculated for all models and runs.

Failed Models

If any models fail to complete properly, they will be listed here.

V. TUTORIAL

A sample dataset is also included in the download of the *Biomod2EZ* script suite (*/Biomod2EZ_SampleData*). This includes a presence/absence table and environmental layers, both of which are required input files to generate species distribution models and ensemble. This data was made with computing power in mind and is small enough to run quite quickly on a personal computer.

Input Files

presabs.csv

This table is made of the following columns: *X*, *X_WGS*84, *Y_WGS*84, and *TestTaxa*. *X* is for individual sample identifiers, *X_WGS*84 is the X coordinate for samples, *Y_WGS*84 is the Y coordinate for samples, and *TestTaxa* contains binary indicators of sample presence (1) and absence (0).

/environmental_layers

This folder contains ASCII rasters for 7 sample environmental layers that can be used to create tutorial species distribution models/ensemble and generate a summary report.

Data Input

1. Download and unzip the Biomod2EZ.zip into your working directory.

2. In R, open 1*_DataInputModelInitiation.R*

3. In the "Data Setup" section, locations where inputs that are needed should replace the existing *XXXXXXXX* placeholders. Each line is annotated with a comment that describes what data needs to be inputted.

    - setwd() is used to set your working directory. This line requires the full filepath to the folder that you are using for your working directory (ex. "D:/R/Biomod2_tutorial"). This folder should contain all of the unzipped Biomod2EZ files.

    - *prstbl* is the identifier for the presence table. Input the filepath to the presence table (presabs.csv) here (ex. "D:/R/Biomod2_tutorial/Biomod2EZ_SampleData/presabs.csv"").

    - *myRespName* is the identifier for the column in the presence/absence

table that contains the species data (in this case, "*TestTaxa*").

- *xname* is the identifier for the column in the presence/absence table that contains the X coordinate or longitude for samples (in this case, "*X_WGS*84").

- *yname* is the identifier for the column in the presence/absence table that contains the Y coordinate or latitude for samples (in this case "*Y_WGS*84").

- *myExpl* is the identifier for a raster stack of all environmental layers used to create the species distribution models. Each line in this stack is for one ASCII raster from the */environmental_layers* folder (ex. "*D:/R/Biomod2_tutorial/Biomod2EZ_SampleData/environmental_layers/annualprecip.asc*").

- *projname* is the identifier for a name for your project (ex. "Biomod2EZ_Tutorial")

Model Parameters, Projections, and Report Generation

1. In R, open */ParametersAndSettings/ParametersAndReportGeneration.R*

2. This script contains all internal settings for model/ensemble creation and preparing parts for the summary report generation. For more information about adjusting settings for Biomod2, see this Biomod Tutorial that uses the same object naming structure: http://www.will.chez-alice.fr/pdf/BiomodTutorial.pdf

3. The last 2 sections of this script deviate from the original Biomod2 framework.

    - Variable Decision Tree - extracts environmental data to points and builds a decision tree for presence/absence from it using the *rpart* package.

    - Generating Report - plots model projections and saves objects that are later included in the summary report.

4. Run all blocks of this script in chunks so that any potential errors will be not be missed. You will end with a report in your working directory called "Biomod2_Report.html" that displays all resulting maps and results and an ASCII raster called "MyEnsembleRaster.asc" that can be used to create maps in external mapping software.

Chapter 7: Concluding Remarks

Summary of Results

This dissertation investigates potential drivers and risks of wildlife infectious disease distributions across Africa including anthropogenic pressures, climate change, and biodiversity. By combining data collected in the field from our research lab with those from recently published studies throughout the region, I was able to effectively assemble a collection of widespread samples for a number of wildlife infectious diseases across the continent. Using modern ensemble distribution modeling techniques, on which I have modified and improved, and high-resolution geospatial datasets, I tested hypotheses aimed to identify drivers of wildlife infectious disease occurrence across Africa.

Chapter 2: Assessing the drivers and transmission risk of wildlife infectious diseases from non-human primates to humans in the Congo Basin

Simian Foamy Virus (SFV) can easily cross over from non-human primates to humans and appears unable to spread within human populations on its own. Thus, I used SFV as an example zoonosis to understand which and how human activities contribute to zoonotic crossover risk. Using SFV as a model, I found that regions exhibiting the highest detected crossover to humans were most closely associated with high levels of bushmeat hunting and close proximity to active logging sites. These two measures were then used to create a map layer encompassing the Congo Basin that shows areas of highest combined crossover risk to humans. Next, the distributions of Ebola Virus, SFV, and Simian Immunodeficiency Virus (SIV)

infections in non-humans primates were modeled across the Congo Basin using environmental predictors and were combined to identify a potential non-human primate zoonotic disease hotspot in northern Gabon and neighboring parts of Cameroon and Republic of the Congo. Models of occurrence for each disease and combined disease hotspot were then compared to a map of human activities that are associated with zoonotic crossover (bushmeat hunting and logging) in order to identify areas that of significant zoonotic risk to humans. Local people in these regions are at higher risk for zoonotic infections because of they rely on hunting animals from the region as a main source of protein (Fa et al., 2003). Bushmeat hunting has become more widespread by the commercial logging industry opening access deep into forests that were previously untouched, and is sustained by demand from bushmeat markets in urban centers where consumption signifies wealth (Wilkie et al., 1999; Fa et al., 2002; Starkey, 2004; Poulsen et al., 2009). Bushmeat hunting and consumption appear to increase risk of zoonotic crossover from non-human primates to humans, and reducing demand from urban centers while educating local people about these risks may be the first steps to prevent future widespread outbreaks of zoonoses.

Chapter 3: Niche divergence between Simian Immunodeficiency Virus (SIV) and Simian Foamy Virus (SFV) in chimpanzees across Equatorial Africa that persists under climate change

Climate change is expected to act most immediately in the tropics, and leaves wildlife facing three potential futures: persisting due to their plasticity, migrating to

track their optimal climatic niche, or risking extinction (Harvell et al., 2009; Cahill et al., 2012). Since climate change is expected to impact host taxa, it is important to consider how their responses may affect the distributions of wildlife infectious diseases over time (Lafferty, 2009). In order to test how disease distributions may change over time, I created a new multi-model climate change dataset, TERRA6-AR5 (Chapter 5)(Sesink Clee, In prep.), that includes measures of temperature, precipitation, and seasonality under all current representative concentration pathways for years 2030, 2050, and 2080 at a global extent. Using this new climate change dataset, I expanded on previous research that identified a potential inverse relationship between geographic distributions of SIV and SFV occurrence in chimpanzees across Cameroon to examine if this inverse relationship was found in chimpanzees in other parts of Central Africa. This new study included the full ranges of the Nigeria-Cameroon Chimpanzee (*Pan troglodytes ellioti*), the Central Chimpanzee (*P. t. troglodytes*), and the Eastern Chimpanzee (*P. t. schweinfurthii*). Using ecological niche modeling techniques, the results suggest that the occurrence of these two viruses appear to remain inversely related across the ranges of *P. t. troglodytes* and *P. t. schweinfurthii*, where high probability of occurrence for one virus coincides with low probability of occurrence for the other virus. This inverse relationship persists under climate change projections, and shows that while their distributions are expected to shift over time, areas of increasing suitability for one virus coincide with decreasing suitability for the other virus. Ultimately, it is important to consider how climate change may affect disease occurrence in wildlife hosts, potentially introducing pathogens to previously uninfected regions.

Chapter 4: Biodiversity and wildlife infectious disease occurrence: understanding the ape Ebola crisis

Previous research has proposed competing hypotheses relating biodiversity to disease occurrence in wildlife: the Dilution Effect and the Amplification Effect (Jones et al., 2008). The Dilution Effect hypothesis suggests that there is an inverse relationship between biodiversity and disease occurrence (Ostfeld et al., 2000; Keesing et al., 2010), while the Amplification Effect hypothesis suggests that biodiversity and disease occurrence are directly related (Lafferty et al., 1999; Randolph et al., 2012; De Thoisy et al., 2016). Using Ebola virus infections in apes, I tested both of these hypotheses using ensemble niche modeling techniques with measures of biodiversity by species richness and evenness for mammals across the study region. Ensemble models revealed an Ebola Virus Disease (EVD) hotspot in northern Gabon that includes high probability of viral presence in both chimpanzees and gorillas that shows an apparent Dilution Effect between predicted Ebola virus occurrence and primate species richness, and an Amplification Effect with bat species richness. Additionally, recent studies suggest that fruit bats in Africa may act as a natural reservoir for Ebola viruses, facilitating spread to wildlife and humans (Leroy et al., 2005; Leroy et al., 2009; Hayman et al., 2010; David et al., 2012; Leendertz et al., 2016a). The inverse relationship between EVD occurrence and primate species richness might occur when areas with high primate species richness saturate the community with low competency primate hosts, limiting exposure and disease spread overall. High primate species richness may also increase interspecific competition for fruits and push apes to rely on more non-fruit

fallback foods such as bark, leaves, flowers, and insects (Harrison et al., 2011), which would reduce contact with fruits that were partially eaten by bats (Leendertz et al., 2016a). Habitat destruction leading to decreases in primates in this region of Africa may leave resilient taxa, such as chimpanzees and gorillas, open to EVD outbreaks. This relationship reveals a case where successful increased protection of primates may increase their diversity and reduce the risk of future EVD outbreaks. The direct relationship between EVD occurrence and bat species richness supports some hypothesis involving these potential natural hosts. Recent studies suggest that multiple bat species may act as competent hosts for Ebola viruses (Huffman et al., 2009; Smith et al., 2013; Olival et al., 2014; Pigott et al., 2014; Banskar et al., 2016), and it is reasonable to expect that areas of high bat species richness might maintain a number of species that are involved in the lifecycle and transmission of Ebola viruses. It appears that the Dilution and Amplification Effect hypotheses cannot be used to completely explain the distributions EVD occurrence in chimpanzees and gorillas, which might be better explained by community composition.

Limitations and caveats of ecological niche modeling

In regions where systematic sampling is difficult due to dense vegetation, difficult terrain, and remote locations, ecological niche modeling can be useful in estimating a taxa's suitable habitat, but due to their correlative nature, these models should be treated with caution (Wiens et al., 2009). A number of caveats should be considered when interpreting the results of ecological niche models. Sampling data

used to create the models are generally incomplete and may lack the resolution to entirely describe a species' ecological niche. Secondly, is not possible to include every variable that defines a species' ecological niche, and unknown factors are likely to contribute to defining their suitable habitat. Furthermore, many models lack detailed information about how target species interact with other organisms including predator-prey relationships, mutualistic behaviors, and interactions between host species communities. Environmental predicting variables often have coarse spatial resolutions, reducing the reliability of models for taxa that have small home ranges or whose habitat is defined on microclimatic levels. Finally, the role of phenotypic plasticity is generally not considered in model construction, and this limits the utility of these models since organisms may be able to tolerate changes over time, allowing them to persist past the extremes of their present niche in the future, leading to pessimistic model projections (Elith et al., 2009; Wiens et al., 2009; Jarnevich et al., 2015). Another potential issue lies in the models themselves, because different modeling techniques can result in varying projected distributions and niche descriptions, even when using the same input data (Araujo et al., 2006). In order to obtain representative models in this dissertation, I used Biomod2 to create ensembles using 11 different modeling techniques weighted by their individual performances (Thuiller et al., 2013) along with the Biomod2EZ data analysis pipeline (Chapter 6)(Sesink Clee et al., 2012).

Broader Impacts

Wildlife infectious diseases and conservation

Understanding how both abiotic and biotic factors can shape the distributions of wildlife infectious disease is an important tool for conservation (Scott, 1988; Wallis et al., 1999; Woodroffe, 1999). This dissertation provides information to bolster understanding about how anthropogenic pressures, climate, and biodiversity can affect wildlife infectious disease incidence and risk across Africa. The forested regions across Central Africa includes some of the highest biodiversity in world, including more than 60 species of primates, 1,500 species of birds, and 50 species of amphibians (Lebreton, 1998; Myers et al., 2000; Oates et al., 2004; Carpe, 2005; Oates, 2011), but less than 4% of these forests fall within protected areas (Myers et al., 2000; Bertzky et al., 2012). The wildlife infectious diseases explored in this dissertation can lead to mortality in their animal hosts, such as Ebola, which has caused large declines in ape populations in Gabon and Republic of the Congo (Formenty et al., 1999; Huijbregts et al., 2003; Leroy et al., 2004b). Future research efforts to ground-truth the models of wildlife infectious disease occurrence presented in this dissertation are needed to confirm model accuracy and provide the necessary means to improve future modeling efforts and boost confidence in the application of these techniques in other regions of the world and with different disease systems.

Ecological niche models have been used in the past to estimate species distributions for conservation action plans and policy suggestions (Kormos et al., 2003; Peterson et al., 2003; Sánchez-Cordero et al., 2005; Papeş et al., 2007). Similarly, distribution

models of disease occurrence in wildlife populations can be used to plan mitigation practices to reduce the risk of disease spread in wildlife populations (Castillo-Chavez et al., 2015). Although many mitigation strategies are purely theoretical (Potapov et al., 2012), there have been cases where eradication efforts of pathogen *in situ* have been successful (Mariner et al., 2012; Wobeser, 2013), most notably in the case of chytridiomycosis fungal infection. This infection has decimated amphibian populations around the world, but researchers were able to eliminate infections in amphibian populations on an island off the coast of Spain using a combination of anti-fungal treatment of tadpoles and environmental disinfection (Bosch et al., 2015). Ecological niche models can be used to target isolated regions of disease occurrence that may be well suited for mitigation attempts before shifting focus to other regions of high estimated disease occurrence.

Zoonotic disease risk to humans

Understanding how biotic and abiotic factors contribute to the distributions of wildlife infectious diseases opens future research opportunities, especially for improving understanding about zoonoses and preventing their spread into human populations. Wildlife infectious diseases with zoonotic potential are a serious concern for public health in areas where people come in close contact with wildlife, such as in Central Africa (Meslin et al., 2000; Chomel et al., 2007). Through this dissertation, an area in northern Gabon and neighboring parts of Cameroon and Republic of the Congo was identified as an important hotspot for a variety of non-human primate pathogens that have the potential to crossover and infect humans

due to increased contact with wildlife in rural areas, high levels of bushmeat hunting, and easy access to remote forests from roads created by the booming logging industry (Chapter 2). Geospatial analyses such as those performed in this dissertation are useful for predicting disease occurrence more rapidly than systematically sampling large geographic regions on the ground (Peterson et al., 2011). Using results from distribution models, researchers can target key areas of highest probability of disease crossover to ground truth the models and assess risks of disease transmission (Guisan et al., 2006).

Bushmeat hunting and consumption have been linked to zoonotic disease crossover, but it has proven difficult to reduce these practices. In rural areas of Central Africa, bushmeat remains a main source of protein for local people. Throughout the region, bushmeat consumption is also ingrained in cultural practices and is likely to persist for generations (King, 1994; Wilkie et al., 1999; Bennett et al., 2000; Cronin et al., 2015; Fa et al., 2016). Over time, bushmeat hunting has shifted from being primarily for sustenance and now includes a profitable business with high demand from urban centers where bushmeat is viewed as a luxury item (Foerster et al., 2012; Bennett, 2015). Mitigation attempts for this system are complex and would require a number of different approaches (Wilkie et al., 1999). Reducing supply of bushmeat by increasing enforcement at markets and checkpoints or implementing a tax may reduce consumption by making alternative sources of protein more affordable in comparison (Fa et al., 2016). With evidence of diseases such as Ebola spreading to humans via bushmeat handling and consumption (Leroy et al., 2004a; Murray et al., 2016), educating local people about the risks of bushmeat in terms of

disease transmission may also be able to reduce demand (Wilkie et al., 1999). A study based in Cameroon found that people who were educated about the risks of disease transmission from wildlife were less likely to consume and handle bushmeat (Lebreton et al., 2006). Efforts to educate people about the risks of bushmeat consumption, combined with proper enforcement of protected areas, where it is currently lacking, may help reduce the demand for bushmeat from urban centers, hindering the spread of zoonoses into highly populated areas while simultaneously conserving wildlife (Lebreton et al., 2006; Fa et al., 2016).

Future research

Research stemming from the results of this dissertation may benefit by expanding to a wider variety of infectious diseases. Some zoonoses infecting non-human primates and other mammals of this region that were not included in this dissertation due to lack of sufficient data for distribution modeling, include monkey pox, anthrax caused by *Bacillus anthracis*, rabies, yellow fever, tuberculosis, and yaws caused by *Treponema pallidum* (Cutler et al., 2010). Assessing more diverse zoonoses may contribute to a more comprehensive assessment of risks to humans and help public health officials focus their efforts and resources. Further research about the future of infectious diseases in other mammals, especially domesticated livestock and those that are hunted for bushmeat such as duiker, would be useful in identifying additional areas where future zoonotic crossover events are likely to occur based on disease presence in animal populations and high levels of human interaction with them. It would also be interesting to investigate the role of the

Dilution and Amplification Effect hypotheses in other disease systems with other systems with transmission routes that do not involve vector, which most literature on this topic is focused on today. Additionally, although accessibility for sampling in Central Africa can be difficult, it is essential for future studies of this nature to increase sampling efforts at large geographic scales to build more reliable and accurate distribution models of wildlife infectious diseases.

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