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Linking social and spatial networks to viral community phylogenetics reveal subtype specific transmission dynamics in African lions

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Summary

1. Heterogeneity within pathogen species can have important consequences for how pathogens transmit across landscapes; however, discerning different transmission routes is challenging.
2. Here we apply both phylodynamic and phylogenetic community ecology techniques to examine the consequences of pathogen heterogeneity on transmission by assessing subtype specific transmission pathways in a social carnivore.
3. We use comprehensive social and spatial network data to examine transmission pathways for three subtypes of feline immunodeficiency virus (FIV_{Ple}) in African lions (*Panthera leo*) at multiple scales in the Serengeti National Park, Tanzania. We used FIV_{Ple} molecular data to examine the role of social organization and lion density in shaping transmission pathways and tested to what extent vertical (i.e., father and/or mother offspring relationships) or horizontal (between unrelated individuals) transmission underpinned these patterns for each subtype. Using the same data, we constructed subtype specific FIV_{Ple} co-occurrence networks and assessed what combination of social networks, spatial networks, or co-infection best structured the FIV_{Ple} network.
4. While social organization (i.e., pride) was an important component of FIV_{Ple} transmission pathways at all scales, we find that FIV_{Ple} subtypes exhibited different transmission pathways at within- and between-pride scales. A combination of social and spatial networks, coupled with consideration of subtype co-infection, was likely to be important

for FIV_{Ple} transmission for the two major subtypes, but the relative contribution of each factor was strongly subtype specific.

5. Our study provides evidence that pathogen heterogeneity is important in understanding pathogen transmission, which could have consequences for how endemic pathogens are managed. Furthermore, we demonstrate that community phylogenetic ecology coupled with phylodynamic techniques can reveal insights into the differential evolutionary pressures acting on virus subtypes, which can manifest into landscape-level effects.

Introduction

Pathogen transmission is a key process for host-pathogen relationships (McCallum 2001), yet the factors that shape transmission are not well understood for most host-pathogen systems. Host heterogeneity in contact rates or susceptibility, for example, are often considered important factors shaping pathogen transmission (VanderWaal & Ezenwa 2016; White, Forester & Craft 2017), particularly for gregarious animals where social organization can affect pathogen spread (e.g., Altizer *et al.* 2003). However, heterogeneities within pathogen populations are less often considered, even though there are clear consequences for how a pathogen transmits, impairs the host, and evolves (e.g., Taylor *et al.* 2008; Ebert 2013; Kerr *et al.* 2015). Different pathogen subtypes can not only have variable health outcomes for the host (Vandegrift *et al.* 2010; Troyer *et al.* 2011), but can have contrasting transmission pathways (e.g., for human immunodefficiency virus (HIV), Taylor *et al.* 2008), and this can lead to different evolutionary pressures on the pathogen (Altizer & Augustine 1997; Ebert 2013; Kerr *et al.* 2015). Minor variation in transmission pathways between strains, for example, for can lead to major changes in pathogen and host dynamics (Kerr *et al.* 2015)

Here, we define "transmission pathways" as how a pathogen is transmitted from host to host, including the spatial and social elements of transmission (i.e., do individuals or groups in close proximity primarily transmit to each other). In particular, analysing pathogen phylogenetic relationships ("phylodynamics" Grenfell *et al.* 2004) can help elucidate transmission pathways by tracing transmission more directly. This enhances the utility of epidemiological models crucial for effective disease management and understanding. Linking phylodynamics to host ecology and behaviour using recent advances in phylogenetic community ecology can reveal the ecological and evolutionary dimensions of pathogen transmission pathways that are difficult to achieve with other techniques, and can help distinguish between alternative pathways for multiple pathogen subtypes across individual to landscape scales.

For retroviruses, molecular data can reveal who has transmitted to whom over timescales relevant for pathogen spread (Biek *et al.* 2015). Feline immunodeficiency virus (FIV) is a directly transmitted retrovirus that is largely host species specific and has limited environmental persistence (Troyer *et al.* 2005; VandeWoude & Apetrei 2006). Even though FIV is rarely the cause of mortality in non-domestic felids, it may have long-term health effects through increased risk of co-infection from other pathogens (Roelke *et al.* 2009, Troyer *et al* 2011). African lions (*Panthera leo*) can be chronically infected with FIV (FIV_{Ple}), with 93% of individuals in the Serengeti National Park, Tanzania infected by one year of age (Packer *et al.* 1999; Troyer *et al.* 2004). FIV_{Ple} can be transmitted both horizontally (via bite and scratch wounds, Brown *et al.* 1994) and vertically (e.g., from parent to offspring), but disentangling the relative contribution of

each transmission pathway in wildlife is difficult. Horizontal transmission is thought to be the dominant pathway for FIV in domestic cats (*Felis catus*, Yamamoto *et al.* 1989) and potentially for African lions, but this is based on seroconversion data from two individual lions (Brown *et al.* 1994). Here we use viral phylogenetic similarity between FIV_{Ple} from parent-offspring pairs to infer vertical transmission (Biek *et al.* 2003). Vertical transmission is likely to be important in systems where pathogen prevalence is high (Lipsitch *et al.* 1995; Biek *et al.* 2003) and theory predicts that vertical transmission can create a transmission bottleneck which can reduce pathogen diversity and virulence (Ebert 2013). While vertical transmission is commonly uniparental (e.g., just from the mother), vertical transmission can be biparental as well (e.g., either through gestation or via sperm, Altizer & Augustine 1997). In host-pathogen systems where prevalence is high and stable, both vertical and horizontal, or 'mixed mode,' transmission may be necessary to maintain equilibrium (Lipsitch *et al.* 1995; Altizer & Augustine 1997; Ebert 2013). Furthermore, different FIV_{Ple} subtypes may have different transmission pathways (Troyer *et al.* 2011).

Extensive data on the social networks, host movement, demographic factors, and viral phylogenetics from 216 individually identified lions from the Serengeti Lion Project (SLP) (Craft 2010) provide a unique opportunity to understand the social and spatial dimensions of FIV_{Ple} transmission pathways. Lions live in social groups (prides) consisting of 1 - 21 related females, their offspring, and a coalition of males (1 - 9) that sometimes reside with multiple prides (Packer *et al.* 2005). When a pride becomes too large, a cohort of females splits off to establish a new pride (VanderWaal, Mosser & Packer 2009). Males disperse large distances from their natal prides and either become resident in another pride or become 'nomads' and do not

maintain territory (Packer & Pusey 1982). The Serengeti population has high FIV_{Ple} diversity, and 50% of all identified FIV_{Ple} subtypes can be found in these lions (FIV_{Ple} A, FIV_{Ple} B and FIV_{Ple} C) (Antunes *et al.* 2008). Approximately 35% of Serengeti lions are co-infected with multiple subtypes (Troyer *et al.* 2011), yet the prevalence of each subtype is variable (12% are infected with subtype A, 69% with subtype B, and 57% with subtype C, Troyer *et al.* 2011); all three FIV_{Ple} subtypes are found throughout the park (Antunes *et al.* 2008).

Data collected from the SLP provides a unique opportunity not only to test whether social organization (who lives with whom) is important for FIV_{Ple} transmission, but also to assess if changes in population density alters transmission pathways. Social organization is important for transmission of Ebola in gorillas (*Gorilla gorilla* , Caillaud *et al.* 2006) and transmission of bacteria in giraffe (*Giraffa camelopardalis*, VanderWaal *et al.* 2014), and it is assumed to play an important role in transmission of FIV_{Ple} in lions, with more transmission expected within prides than between prides (Fig. 1a/b, i.e., a pride effect, Troyer *et al.* 2004). However, landscape and habitat can also shape patterns of social organization by either clustering or dispersing individuals and thus driving transmission. For example, certain habitats with limited resources can cluster individuals and therefore increase the frequency of transmission events between different social groups, thus reducing the effect of social organization (Fig. 1c, Chiyo *et al.* 2014). The SLP study area includes prides that occupy woodlands, plains, and the boundary of both habitats, with much higher pride density in the woodlands as compared to the plains (Mosser *et al.* 2009). Lion density was also altered by an outbreak of a pathogen with high mortality, which may have affected FIV_{Ple} transmission by reducing the number of contacts between individuals. The Serengeti lion population was reduced by ~30% due to a canine

distemper virus (CDV) outbreak in 1993-1994 (Roelke-Parker *et al.* 1996). FIV_{Ple} genetic material was collected from Serengeti lions in pre-CDV (1984-89) and post-CDV (1994-99) time periods, and as such we have a unique opportunity to analyse the effect of the CDV epidemic on transmission patterns for multiple subtypes of FIV_{Ple} (Fig. 1c).

The SLP data can also provide insights into the extent that horizontal, vertical, or mixed mode transmission explain within-pride FIV_{Ple} dynamics (Fig. 1d/e), and how these transmission routes scale up to the landscape scale (Fig. 1f/g). Between-pride transmission may result from a series of local contacts whereby transmission is through aggressive interactions (horizontal transmission) between neighbouring prides (Fig. 1f); aggressive interaction between individuals competing for territory has been suggested to be a major source of FIV infection of solitary mountain lions (*Puma concolor*) (Wheeler, Waller & Biek 2010). Between-pride contact is determined in part by whether two prides are neighbours geographically (Craft *et al.* 2009, 2011), but it is not known if pride neighbour status or other spatial proxies for local contacts, like distance between prides or territory overlap, translate into a risk for FIV_{Ple} transmission (Fig. 1f). Alternatively, FIV_{Ple} transmission may be a mix of localized and long-distance jumps across the landscape determined by, for example, by male immigration (Fig. 1g). Furthermore, as interactions between strains has been shown to be important for pathogen persistence and the evolution of virulence (e.g., Turner & Chao 1999; Susi *et al.* 2015; Kerr *et al.* 2015; Cressler *et al.* 2016), transmission of one subtype of FIV_{Ple} from one pride maybe determined by the prevalence or diversity of other subtypes in another pride (Fig. 1h). These interactions could be antagonistic (e.g., competition for host resources) or facilitative (e.g., subtypes cooperate or co-transmit, see Cressler *et al.* 2016 for a review of the topic), yet empirical evidence of either mechanism is scarce, particularly at the population level (Cressler *et al.* 2016)

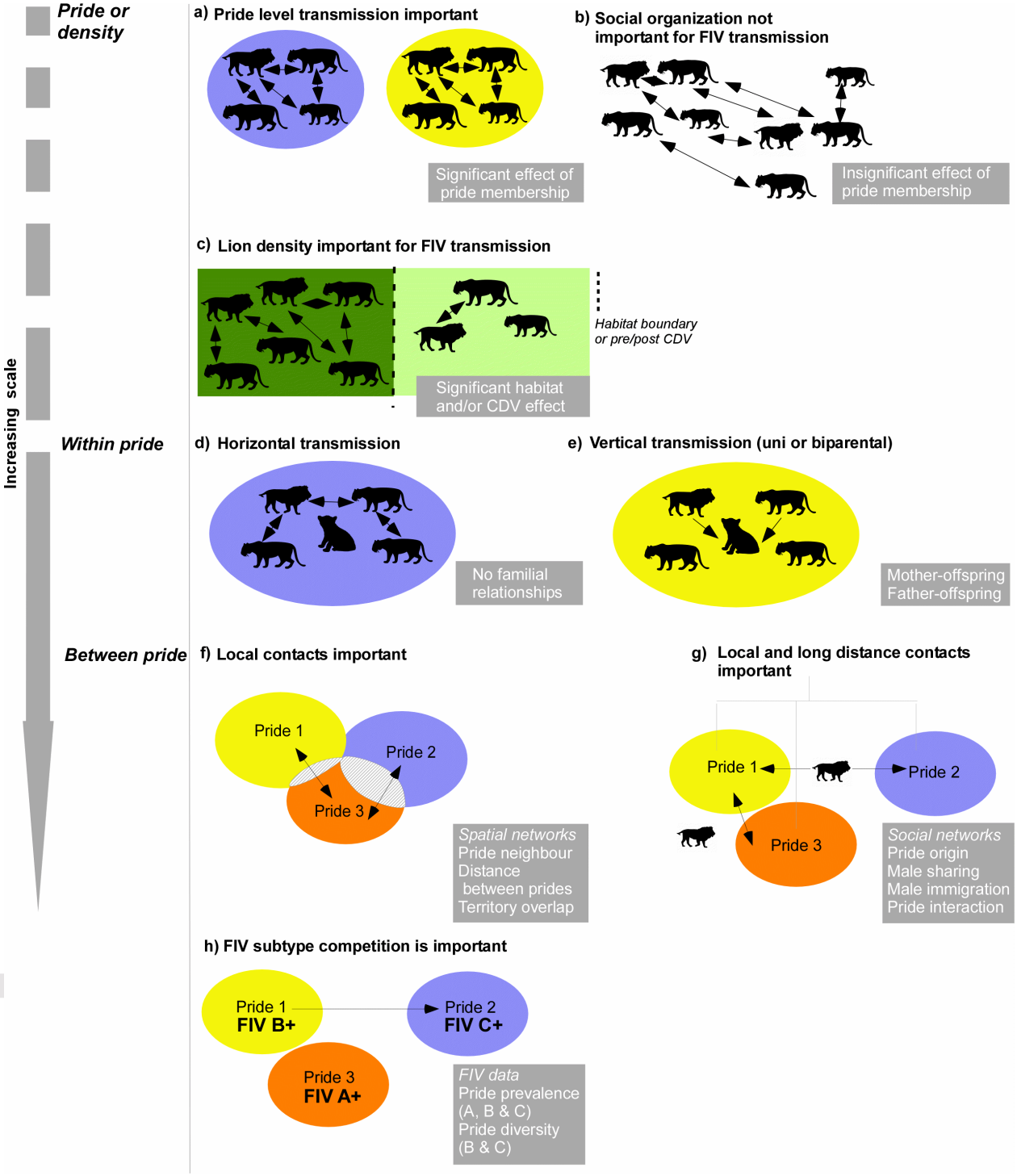

Fig. 1. Transmission pathway hypotheses for FIV_{Ple} across scales a-c): pride or density affects transmission, d-e): within-pride transmission pathways, and f-h): between-pride level transmission pathways. Transmission pathways are likely to be linked between scales. Black

arrows indicate directionality of transmission, ovals indicate pride territories, and coloured squares reflect differences in habitat or densities before and after a disease outbreak. See Table 1 for definitions of spatial and social networks. '+' in h) indicates that this subtype was more prevalent or phylogenetically diverse in each respective subtype. Therefore, if pride 1 has a large diversity or high prevalence of FIV_{Ple} B, those members of pride 2 that are infected with FIV_{Ple} C might be more likely to get FIV_{Ple} B.

Here we explore the role of pathogen heterogeneity on transmission pathways across scales using a novel mix of phylogenetic and community ecology approaches (Fig. 1). Specifically, for each subtype we ask: *i*) What extent does lion pride membership and lion density shape FIV_{Ple} transmission; *ii*) What contribution do horizontal, vertical, and mixed mode pathways make in transmission of FIV_{Ple} within prides; and *iii*) What spatial networks, social networks and subtype co-infection patterns best explain FIV_{Ple} transmission pathways between prides? In addressing these questions, we provide one of the first examples of a community ecology analytical approach integrating key epidemiological parameters into an investigation of multi-scale viral transmission pathways (Johnson, de Roode & Fenton 2015).

Materials and Methods

Study system

The study population included lions from 16 prides in a 2000 km^2 area of the Serengeti National Park (Fig. 2a). The vegetation of the Serengeti National Park consists of woodlands to the north and west and plains to the southeast. We utilized data on individually identified lions, including FIV_{Ple} exposure, pride name, pride location, contacts with other prides, and familial

relationships, based both on SLP observational (1966-1999) and genetic data pre- and post-CDV outbreak (1984-89 and 1994-99) (Craft 2010).

Genetic data

We analysed a 337 bp region of the FIV_{Ple} *pol reverse transcriptase (pol RT)* gene from 216 individual lions. Details regarding the amplification, sequencing, and sequence alignment can be found in Troyer *et al.* (2004). The FIV_{Ple} *pol RT* gene is one of the most stable in the FIV_{Ple} genome (Troyer *et al.* 2004) making it a suitable region to trace FIV_{Ple} transmission. Of the 216 Serengeti FIV_{Ple} sequences , 68 were included in Troyer *et al.* (2004) (GenBank accession numbers AY549217 to AY549304, AY552614 to AY552683, and AY552684 to AY552748) with the remainder sequenced for strain typing without phylogenetic analysis from Troyer *et al.* (2005) (GenBank accession numbers AY878208 to AY878235). The combined dataset yielded sequences for three FIV_{Ple} subtypes: A: 32 sequences; B: 149 sequences; and C: 117 sequences. OTUs (operational taxonomic units) were delimited based on a 95% genetic similarity threshold commonly applied to retroviral genetic datasets (Yin *et al.* 2012; Emerson *et al.* 2013) using Geneious Version 8.1.5 (Kearse *et al.* 2012). OTUs were named numerically for ease of identification (e.g., FIV_{Ple} B1, B2).

Phylogenetic reconstruction was performed on individual sequences and for OTUs using MrBayes (Huelsenbeck & Ronquist 2001) based on a MUSCLE alignment using the default settings (Edgar 2004). We used a GTR+gamma evolutionary model (considered most appropriate using jModelTest 2, Darriba *et al.* 2012) and a MCMC chain length of 10 000 000 (burn in at 100 000). For each subtype, pairwise patristic distances (sum of phylogenetic branch lengths) were calculated based on the maximum clade credibility (MCC) consensus tree (Figs. S1-3).

Pride or density analysis

To test for the importance of pride membership and lion density in shaping FIV_{Ple} phylogenetic relationships, we applied a three-way nested factorial permutation ANOVA (PERMANOVA) (Anderson 2001) on the patristic distance matrix for each subtype. PERMANOVA is a flexible non-parametric routine capable of analysing any symmetric distance matrix (Anderson 2001). Pride, habitat, and CDV outbreak were treated as fixed factors; and habitat was nested within-pride. 'Habitat' (Table 1, i.e., woodland or plains) was assigned to prides based on territory data (70% kernel) from 1966-99. To account for non-independence of samples (where the same prides were sampled in both decades), the analysis accounted for repeated measures by excluding the highest order interaction (Anderson, Gorley & Clarke 2008). As there was unequal sampling across prides, Type III sums of squares (SS) were used, 9999 permutations calculated, and a Monte Carlo test used to determine significance. Type III SS account for unbalanced designs by fitting each term to the model only after accounting for all other terms, and as a trade-off provide conservative effect estimates (see Anderson *et al.* 2008). Pseudo R_2 were calculated for each PERMANOVA model following Kelly *et al.* (2015). Due to the small numbers of FIV_{Ple} samples taken from some prides, the assumption of homogeneity of multivariate dispersions could not be reliably tested (Anderson 2004). To help overcome this limitation, canononical analysis of principal coordinates (CAP) (Anderson & Willis 2003) was performed on significant terms to visualize effect size and to help confirm PERMANOVA results using cross-validation. CAP model cross-validation was performed using the 'leave-one-out' procedure to assess the misclassification error of assigning individual FIV_{Ple} sequences to their respective groups based on patristic distance (see Anderson & Willis 2003 for details). All of the above analyses were

conducted in PRIMER- E PERMANOVA+ software (Anderson *et al.* 2008) unless otherwise stated.

To analyse if viral diversity of FIV_{Ple} B and C was changing pre/post CDV outbreak, Bayesian skyline plots were generated based on the birth dates of lions from each sample using BEAST 1.8 (Drummond & Rambaut 2007) using a piecewise linear skyline models with 5 groups (see Drummond *et al.* 2005). Bayesian skyline plot models (Drummond *et al.* 2005) use coalescent-based inference methods correlating genetic diversity to Wright–Fisher population models, see de Silva, Ferguson & Fraser (2012). Bayesian viral skyline plots are calculated independently of lion population size estimates so, to enable direct comparison with the host population, we overlaid monthly lion population size estimates for the SLP area (1966-99). We did not analyse FIV_{Ple} A genetic diversity (or within or between pride transmission pathways) due to insufficient data because of the low prevalence. A visual summary of the methods for the pride and density analyses is show in Figure 2a.

Within-pride transmission

To assess whether any pride effect was due to transmission between pride mates or between parent-offspring pairs, we analysed FIV_{Ple} patristic distance between 179 parent-offspring pairs (Fig. 2b). Parent-offspring pairs which were considered 'confirmed' were based on genetic parentage analysis (see Packer *et al.* 1991). 'Candidate' fathers were identified as the pride's resident male lions when a given set of cubs was conceived, and 'candidate mothers' nursed the offspring. FIV_{Ple} patristic distance for confirmed and candidate mother-offspring pairs and confirmed and candidate father-offspring pairs were tested using a one-way factorial PERMANOVA for each FIV_{Ple} subtype (Fig 2b). As we assumed that pride membership was

important for FIV_{Ple} patristic distance, pride was added as a covariate to the models. Type I (sequential) sums of squares were used in this routine, as the covariate is fitted first then followed by the parent-offspring factor (Anderson *et al.* 2008). Each parent-offspring relationship was coded as a factor by assigning a unique number. Only prides with > 1 parent-offspring relationship were included in the analysis to account for the pride effect. For FIV_{Ple} B, 36 confirmed and candidate mother-offspring and 46 father-offspring dyads were included in analysis. For FIV_{Ple} C, there were 50 mother-offspring pairs and 47 father-offspring pairs. To determine the significance of offspring assignment compared to the null model (random assignment), PERMANOVA and associated diagnostic tests were performed as described above.

Between-pride transmission

To generate the FIV_{Ple} co- occurrence network (hereafter referred to as the FIV_{Ple} network), we built a contingency table that described the occurrence (presence/absence) of OTUs across prides (Fig. 2c). This incidence matrix was of size m*n, where m was the number of prides and n was the number of OTUs. We then created an adjacency matrix (m*m) that described OTU occurrence in prides by multiplying the incidence matrix by its transposed form. One-half of the resulting matrix provided the information required to build a network that described the number of OTUs shared by pairs of prides. Next, we evaluated which prides showed similar patterns of infection by FIV_{Ple} B and C OTUs. These sets of highly connected pride communities, or clusters, were defined using a "greedy" approach (Clauset, Newman & Moore 2004). This approach optimized the classification of prides in clusters in the following ways: *i*) by maximizing the modularity index that reflects the ratio of OTUs shared among individuals both within clusters and between clusters; and *ii*) by assigning prides to the smallest number of clusters possible. The adjacency matrix was obtained from the incidence matrix using the

graph.incidence and the *bipartite.projection* functions using the igraph library in R (Csárdi & Nepusz 2006). The classification analysis was performed using the fast-greedy community function, and graphical output was produced using the *tkplot* function.

Network predictor variables

In total, 14 social, spatial, and FIV_{Ple} co-infection variables were used as potential predictors of between-pride transmission (Table 1). Variables that fluctuated over time and where historic events could affect the observed FIV_{Ple} network (i.e., male immigration, male sharing and territory overlap) were averaged from 1966-99. As FIV_{Ple} transmission events lead to chronic lifelong infections, and Serengeti male lions can live up to 13 years and a female up to 20 years, a between-pride transmission event many years in the past may still leave a signature in the observed FIV_{Ple} network. For example, if two prides shared males consistently throughout the 34 year period (and this led to FIV_{Ple} transmission events), yet did not during the two FIV_{Ple} sampling periods, averaging the impact of shared males over time allowed us to account for longer term trends in the model. Conversely, variables that were relatively stable through time (distance between pride and pride neighbour relationships) were summarized across a two-year period (1987-89). Pride interaction frequencies were calculated over a 2-yr period (1985-1987). See the last panel of Fig 2 for a summary for the time frame of each data set.

'Distance between prides' was approximated as the Euclidean distance between the centroids of each pair of pride territories, as calculated by 70% kernel estimates from VHF tracking data over a 2 year period (Mosser *et al.* 2009) (Table 1). As prides may be far apart, yet may still be neighbours in poor habitat, 'pride neighbour' was also calculated. If pride territory dyads (defined by a 70% kernel) were not separated by any other territory, an index of 1 (e.g., neighbour) was assigned; an index score of 2 was given for dyads that were separated by only

one other pride; and a score of 3 was assigned to dyads that were separated by ≥ two prides (Table 1) (Craft *et al.* 2011). As another proxy of interaction risk, between-pride 'territory overlap' was calculated from the average percentage of 1×1 km grid cells that each pride-pair shared compared to the total cells occupied by both prides; overlaps were calculated every two years from 1966-99 based on 75% home-range kernel estimates (VanderWaal *et al.* 2009). We calculated pairwise 'pride origin' relationships as the number of years prior to 1992 (the midpoint of our sampling period) following an observed split from the parent pride (Fig. S4). If prides did not share a common ancestor, a value of 100 was given. We calculated 'male sharing' and 'male immigration' variables using the number of occasions when a male coalition was simultaneously resident in the two prides, and counts of male immigration between pairwise prides from 1966-1999. 'Pride interaction' was calculated from the number of contacts between prides (defined as moving within 200m of each other) from 1985-87, and correcting for observational bias (Craft *et al.* 2009, 2011).

FIV co-infection predictor variables

Prevalence of each subtype and of subtype co-infections was calculated for each pride (Table 1). For FIV_{Ple} B and C we characterized OTU phylogenetic diversity (PD) using the nearest taxon index (NTI, Webb *et al.* 2002), net relatedness index (NRI, Webb *et al.* 2002), and phylogenetic species variability (PSV, Helmus *et al.* 2007) (Table 1). We used NTI for the between-pride models as there were strong correlations between each metric ($\rho > 0.75$). NTI is calculated by comparing standardized effect sizes of the mean nearest taxon distance (MNTD) using the formula: $NTI = -(MNTD_{obs} - mean(MNTD_{null})/sd\ (MNTD_{null}))$, where the null model was generated by randomizing the tip labels of the OTU phylogeny (n = 9999). All PD indices were calculated using the R package 'pez' (Pearse *et al.* 2015).

Table 1. Model variable definitions and calculation summary from the empirical data.

Predictor variables	Time period	Variable definition	Data type	Citation
Habitat	1966-1999	Dominant habitat type each pride occupied	Binary (Woodland/Plains)	SLP data
Distance between prides	1987-89	Location of centroids of each territory	Latitude/Longitude	(Mosser *et al.* 2009)
Pride neighbour	1987-89	1: Direct neighbour, 2: 2nd tier neighbour, 3: 3rd tier	Matrix	(Craft *et al.* 2009, 2011)
Territory overlap	1966-1999	Average percentage of shared territory between prides	Matrix	(VanderWaal *et al.* 2009)
Pride origin	1966-1993	Number of years since each pride split from a 'parent' pride*	Matrix	SLP data
Male sharing	1966-1999	Male coalitions that were resident in multiple prides	Matrix	SLP data
Male immigration	1966-1999	Males resident in a non-natal pride	Matrix	SLP data
Pride interaction	1985-1987	Pride interaction frequencies	Matrix	(Craft *et al.* 2009, 2011)
FIV_{Ple} A prevalence	1984-1999	Prevalence of subtype in each pride	Percentage	SLP data
FIV_{Ple} B prevalence	1984-1999	Prevalence of subtype in each pride	Percentage	SLP data
FIV_{Ple} C prevalence	1984-1999	Prevalence of subtype in each pride	Percentage	SLP data
FIV_{Ple} B PD	1984-1999	NTI of subtype in each pride	Numeric	SLP data
FIV_{Ple} C PD	1984-1999	NTI of subtype in each pride	Numeric	SLP data
FIV_{Ple} co-infection prevalence	1984-1999	Prevalence of subtype co-infections in each pride.	Percentage	SLP data

*: See electronic supplementary material S4 for details, PD: phylogenetic diversity, NTI: nearest taxon index. Variables that were likely to vary through time were averaged over longer periods (e.g., 1966-99), whereas variables more stable over time were averaged over shorter periods of time (e.g., 1987-89). Pride interaction was averaged 1985-87 due to data availability.

Modelling the between-pride FIV_{Ple} network

Between-pride transmission pathways were analysed by constructing a co-occurrence network based on prides that shared the same FIV_{Ple} molecular OTUs, and modelling which spatial or social network or co-infection predictor (see Table 1 for predictor details) was the most important in explaining the FIV_{Ple}network (hereafter referred to as the FIV_{Ple} network) using generalized dissimilarity modelling (GDM) (Ferrier *et al.* 2007). We standardized the weighted FIV_{Ple} adjacency matrix using the formula: $ds_{FIVple} = 1 - (\frac{d_i}{max(d_{ij})})^2$ where *d* is pairwise distance. To enable direct comparison, we standardized each predictor matrix the same way. Predictor correlation was overall < 0.70. We then employed GDM to identify the separate predictors that best explained the FIV_{Ple} network structure for each subtype (excluding predictors related to that subtype, i.e., prevalence and diversity of that particular subtype). Otherwise, for each subtype the predictor sets used were identical. GDM is a nonlinear, multivariate extension of Mantel correlation and regression techniques and is commonly used for analysing and predicting patterns of dissimilarity. Unlike Mantel tests or other regression techniques, GDM accounts for nonlinear relationships between response and predictor variables by fitting splines to the predictor variables themselves, rather than to a distance matrix based on predictor variables (Ferrier *et al.* 2007). Specifically, GDM uses GLMs (Generalized Linear Models) to model observed FIV_{Ple} network in the form of:

$$-ln(\,ds_x) = \,a_0 + \,\sum_{p=1}^{n} |\, f_p(x_{pi}) - f_p(x_{pj}) \,|$$

where *i* and *j* are prides, a_0 is the intercept, *p* is the number of covariates and $f_p(x)$ are I-spline transformed versions of the predictor network variables (see Ferrier *et al.* 2007; Fitzpatrick & Keller 2015 for further details). We performed model selection using backward elimination and

employed permutation tests (n = 99) to test for significance (Ferrier *et al.* 2007; Fitzpatrick *et al.* 2011). The model that retained the highest deviance (±2% deviance explained) with the smallest number of predictors was reported.

To further explore the roles that co-infection patterns could play on between-pride transmission we performed probabilistic co-occurrence analysis (Veech 2013) to test for either positive or negative associations between FIV_{Ple} OTU pairs by applying the R package 'coocur' using the default settings (Griffith, Veech & Marsh 2016).

As this study consisted of a large number of tests, to reduce the potential for type I error all P values reported are after false discovery rate (FDR) adjustment for multiple comparisons (Benjamini 1995). Figure 2 provides a summary of the data sets and tests used to answer the questions at each scale.

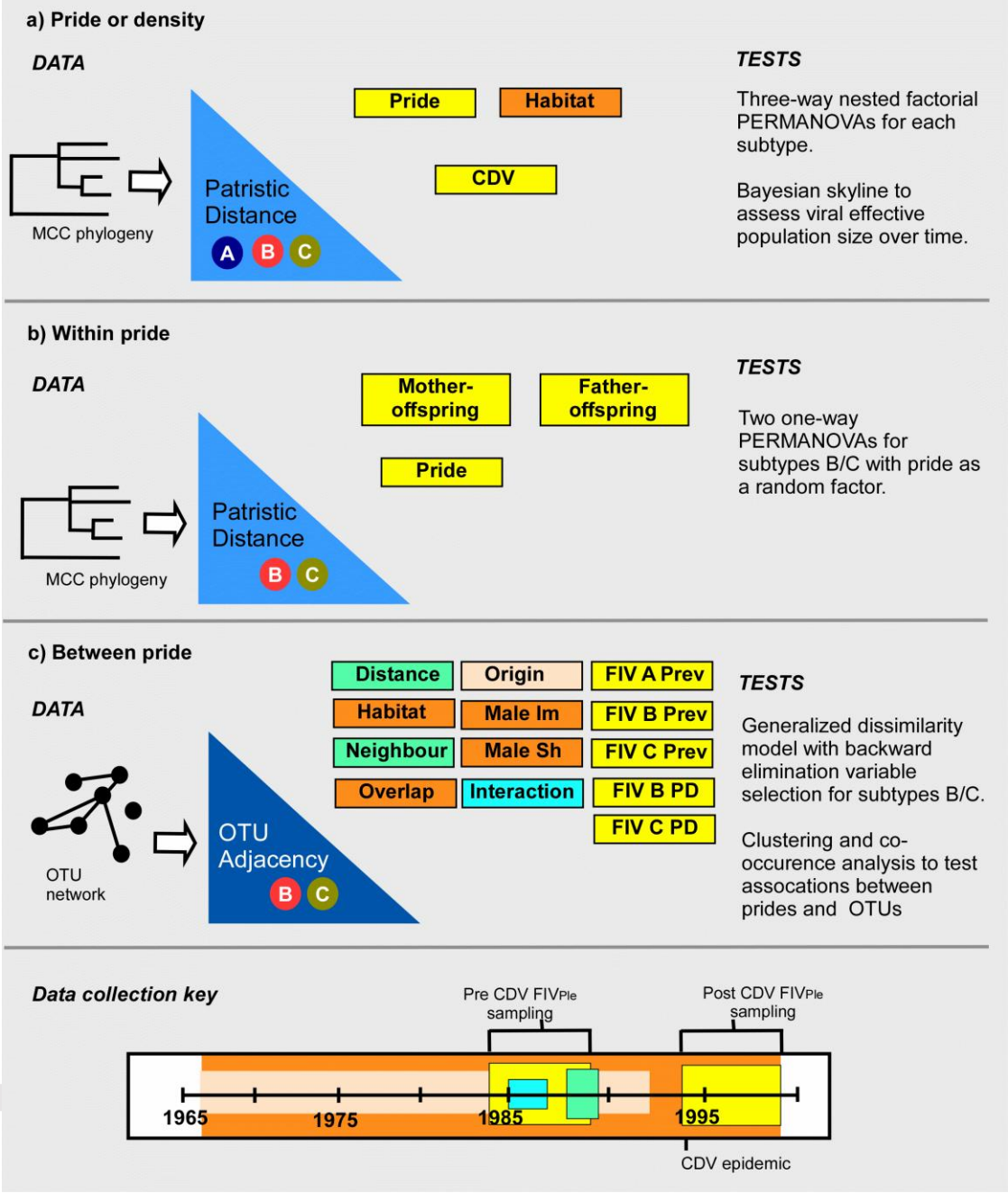

Fig. 2. Schematic summarizing the data sets and tests used in this study (see Table 1 for variable definitions). Variables (represented as rectangles) used in each test are colour coded based on when the data was collected (see the key in the bottom panel). Triangles reflect pairwise matrices. MCC: Maximum clade credibility, CDV: Canine distemper virus, Distance: Distance

between prides, Male Im: Male immigration, Male Sh: Male sharing, Prev: Prevalence, PD: Phylogenetic diversity.

Results

Pride or density impacts on transmission

We found that pride membership was a significant factor explaining relatedness for two of the three FIV_{Ple} subtypes (FIV_{Ple} B: pseudo $R^2 = 0.21$, $P < 0.001$; FIV_{Ple} C; pseudo $R^2 = 0.34$, $P < 0.001$) (Table 2). For FIV_{Ple} A, there was a significant pride × CDV interaction ("P x CDV"; pseudo $R^2 = 0.14$, $P = 0.008$). According to post-hoc pairwise contrasts, the pride effect for FIV_{Ple} A was significant post-CDV outbreak when lion densities were reduced (Table S1), though due to uneven sampling, tested pride-pairs were not the same in each time period. There was no significant CDV effect for either subtype B or C (Table 2), yet there was a weak pride × CDV trend for FIV_{Ple} B (pseudo $R^2 = 0.08$, $P = 0.0113$), with pairwise tests indicating that the pride effect was stronger post-CDV (Table S2). There was no corresponding post-CDV effect for FIV_{Ple} C (Table S3). Measure of allocation success for sequences to each pride from the CAP models were much higher than expected by chance which helps confirm that the PERMANOVA results were robust . The CAP models could correctly allocate sequences to prides 38.89% (FIV_{Ple} A, null = 12.5%), 39.05% (FIV_{Ple} B, null = 5.88%), 48.04% (FIV_{Ple} C, null = 7.14%) of the time (see Fig. S5).

Despite lion population fluctuations through time, Bayesian skyline plots demonstrated a small declining trend in genetic diversity of FIV_{Ple} B since the 1960s, but not for C (Fig. S6). In particular, the CDV epidemic had no apparent effect on FIV viral genetic diversity for these two subtypes, despite the large effect on the population size of the host during this period.

Within-pride transmission

Mother-offspring pairs were more likely to have closely related FIV_{Ple} compared to other dyads within the same pride, but the strength of mother-offspring and father-offspring relationships varied by FIV_{Ple} subtype. For FIV_{Ple} B, the PERMANOVA on patristic distance indicated that there was weak mother to offspring trend (pseudo $R^2 = 0.54$, $P = 0.073$), but not for father-offspring pairs even though the trend was positive (pseudo $R^2 = 0.36$, $P = 0.15$, Table 2). CAP models for FIV_{Ple} B confirmed these results (Fig. S7 with allocation success of 12.76% (null = 8.33%, $P = 0.065$) and 20.00% (null = 7.14%, $P = 0.415$) respectively). In contrast, there was a strong mother- and father-offspring effect on transmission of FIV_{Ple} C (pseudo $R^2 = 0.56$, $P = 0.005$ and $R^2 = 0.58$, $P = 0.002$ respectively, Table 2), both fully supported by their respective CAP model (Fig. S7 with allocation success of 28% (null = 5.26%, $P < 0.001$) and 32.06% (null = 6.67%, $P < 0.001$), respectively).

Table 2. Summary of results from pride or density and within-pride analyses for each FIV_{Ple} subtype.

Pride or density?	**FIV_{Ple} A**	**R^2+**	**F**	**$P_{(MC)}$**	**FIV_{Ple} B**	**R^2+**	**F**	**$P_{(MC)}$**	**FIV_{Ple} C**	**R^2+**	**F**	**$P_{(MC)}$**
PERMANOVA	**Pride**	**0.59**	**5.08**	**<0.001**	**Pride**	**0.21**	**1.69**	**<0.001**	**Pride**	**0.34**	**5.11**	**<0.001**
	Habitat (pride)	0.03	2.00	0.165	Habitat (pride)	0.05	1.08	0.394	Habitat (pride)	<0.01	0.56	0.537
	CDV outbreak (pre or post)	0.05	0.69	0.71	CDV outbreak (pre or post)	0.04	1.03	0.377	CDV outbreak (pre or post)	<0.01	1.66	0.208
	P × CDV*	**0.14**	**4.39**	**0.008**	P × CDV*	0.08	1.33	0.113	P × CDV*	<0.01	1.32	0.254
Within pride												
PERMANOVA	N/A				Mother-offspring	0.54	1.40	0.073	**Mother-offspring**	**0.56**	**2.30**	**0.005**
	N/A				Father-offspring	0.36	1.35	0.15	**Father-offspring**	**0.58**	**8.29**	**0.002**

*$P_{(MC)}$ = P value based on Monte Carlo permutations; significant predictors are in bold; +: pseudo R^2; *: before and after the CDV outbreak; P×CDV: Pride × CDV outbreak (pre or post) interaction.*

Between-pride transmission

From the 216 lions sampled, 38 FIV_{Ple} OTUs were identified, and of these, 22 OTU consisted of individual sequences (i.e., these individual sequences were > 5% different than all other sequences). FIV_{Ple} B had the greatest diversity and number of OTUs (30), followed by subtype C (7), and then A, which consisted of only one OTU. The FIV_{Ple} B and C networks varied substantially. FIV_{Ple} B formed two distinct transmission clusters with woodland prides to the east forming a cluster compared to the western woodlands and plains prides (Fig. 3b). In contrast, FIV_{Ple} C formed three distinct clusters, yet there was no clear spatial signature explaining cluster patterns (Fig. 3b).

The variables s that strongly correlated with the FIV_{Ple} B and C networks also differed. The best model explaining FIV_{Ple} B network structure included distance between pride ($P < 0.001$, for change in deviance for all predictors see Fig. 3c) and prevalence of FIV_{Ple} A ($P = 0.02$, for change in deviance see Fig. 3c). In other words, prides were more likely to have similar B OTUs if they were close in space and did/not have A. The best model explaining FIV_{Ple} C network structure included co-infection FIV_{Ple} prevalence ($P = 0.02$) and prevalence of FIV_{Ple} B ($P = 0.04$, for change in deviance see Fig. 3c) and male immigration ($P > 0.001$). In other words, prides were more likely to have similar C OTUs if they shared connections via male immigration and had a relatively high prevalence of FIV_{Ple} co-infection and a low prevalence of subtype B. Even though distance between pride was also a significant predictor of FIV_{Ple} C network structure ($P = 0.03$), it was only a weak predictor as the amount of deviance explained was low (Fig 3c). In other words, prides were more likely to have similar C OTUs if they had a relatively high prevalence of FIV co-infection and had a high prevalence of B. The best GDM model for

each subtype B and C accounted for similar amounts of deviance (FIV_{Ple} B: 66.39., $P < 0.001$; FIV_{Ple} C: 63.55, $P = 0.01$). Phylogenetic diversity of FIV_{Ple} B and C were not important predictors of the either network.

Further analysis of these pride-level co-infection patterns revealed that prides with low FIV_{Ple} A prevalence were often outliers in the FIV_{Ple} B network (Fig. S8a). Similarly prides with high prevalence of FIV_{Ple} B and low FIV_{Ple} co-infection prevalence were often significant outliers in the FIV_{Ple} C network (Fig. S8b-c). The co-occurrence analysis showed that OTU occurrences were not random since FIV B1 was positively associated with A1, and C1 was positively associated with B5 and B2 (Fig. S8d).

A schematic of the results (Fig. 4) provides a summary of different transmission pathways for each subtype across all scales.

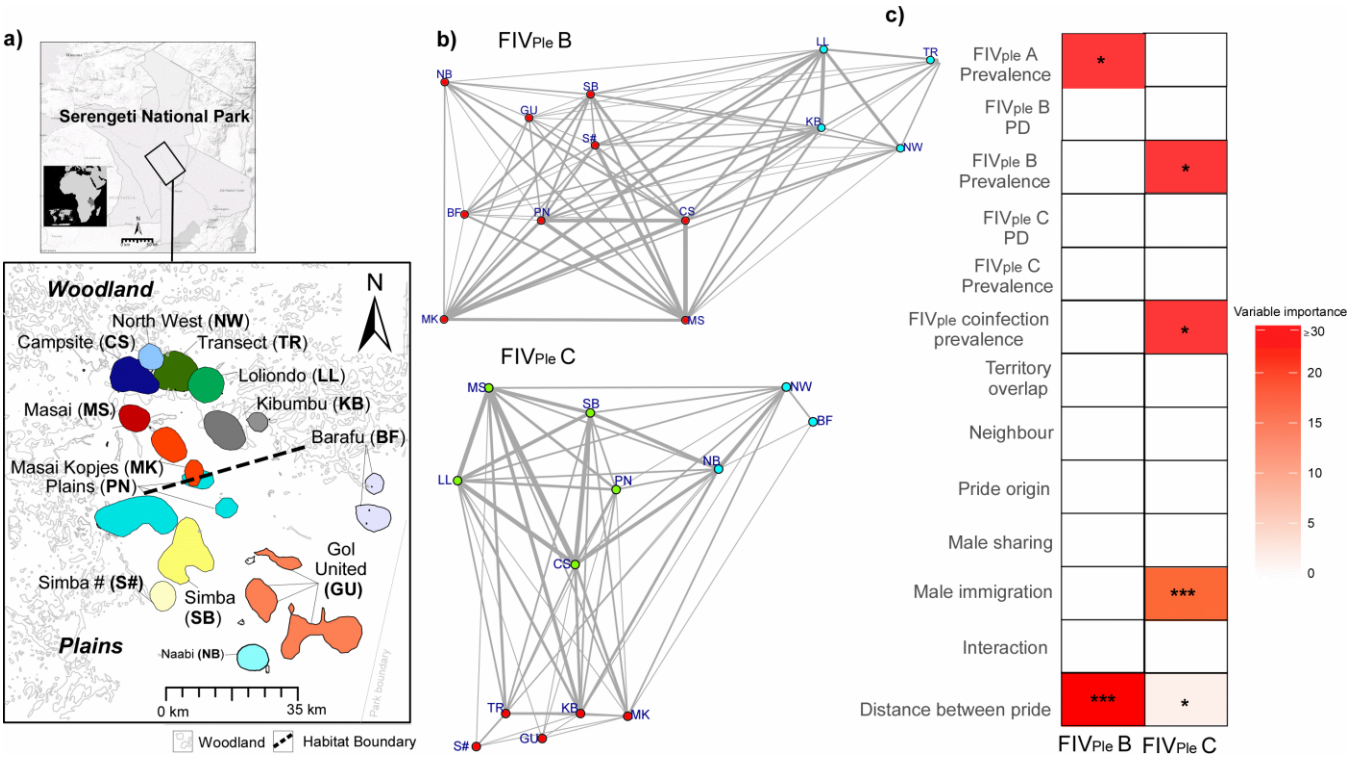

Fig. 3. a) Map of the study area illustrating the spatial distribution of pride territories (based on the 1986-87 70% territory kernel) with colours representing different prides. b) Representation of pride FIV_{Ple} B and C networks where nodes are prides and edges reflect shared FIV_{Ple} OTUs. Distinct communities of prides displaying similar infection patterns were identified using a "greedy approach"(Clauset *et al.* 2004); communities are plotted in different colours. Edge thickness is proportional to the number of OTUs shared by pride pairs. c) Generalized dissimilarity model (GDM) results showing the social and spatial network variables that are most important and significant in explaining FIV_{Ple} B or FIV_{Ple} C network structure. Variable importance (red gradient) was calculated by comparing the change in deviance explained between a model fit with and without that variable (see Fitzpatrick *et al.* 2011). Significance was determined using permuted P values. ***: $P < 0.001$, *: $P = 0.01 - 0.05$.

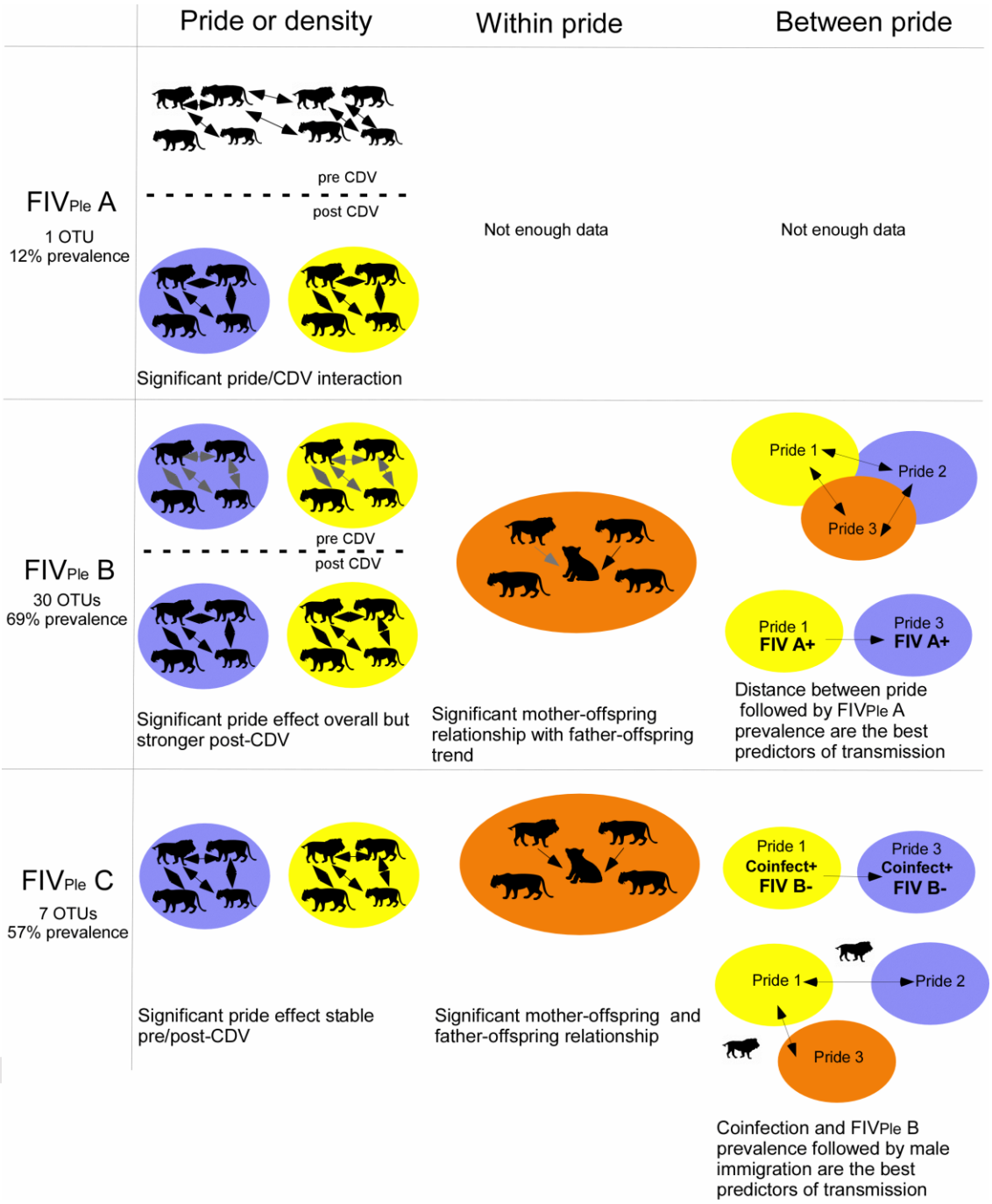

Fig. 4. Schematic summarizing results for each FIV_{Ple} subtype at each scale. See Table 1 for variable details. Overall prevalence estimates for each FIV_{Ple} subtype are from Troyer *et al.* (2011). Black arrows indicate likely directionality of transmission ($P < 0.05$), while ovals

indicate pride territories. Grey arrows indicate when statistical tests showed a trend (P = 0.05 - 0.1).

Discussion

By applying a novel phylogenetic community ecology approach that linked viral phylodynamics to spatial and social networks, we demonstrated that pathogen heterogeneity can lead to subtype specific transmission pathways across scales. FIV_{Ple} likely spreads through a mixture of local and long distance transmission events as seen by parent-offspring transmission and long distance movements via male immigration, but also shaped in part by competition between subtypes. However, the mechanics of FIV_{Ple} transmission varied by subtype at all scales. Our findings indicate: (*i*) social organization shaped viral transmission though this varied by subtype; (*ii*) mixed-mode transmission appeared to be important for FIV_{Ple} B and C subtypes, but the relative contribution of vertical and horizontal mechanisms differed between subtypes, and (*iii*) prides that are linked via male immigration and have relatively high prevalence of FIV_{Ple} co-infection (but relatively low FIV_{Ple} B prevalence) are more likely to be strongly connected in the FIV_{Ple} C network, whereas prides in closer spatial proximity with high prevalence of FIV_{Ple} A were more likely to be strongly connected in the FIV_{Ple} B network.

Although social organization was critical for FIV_{Ple} transmission dynamics, the pride effect on FIV_{Ple} A transmission was only significant when lion densities were reduced after the CDV epidemic; FIV_{Ple} B showed a similar trend. Given a relatively stable genetic diversity of FIV_{Ple} B, there was no evidence that the number of transmission events declined with the reduction in host density after the CDV outbreak. The decrease in lion numbers post-CDV may have reduced

levels of between-pride competition and hence inter-pride conflicts, thereby limiting FIV_{Ple} transmission to within-pride events for FIV_{Ple} A (and to a lesser extent FIV_{Ple} B). For FIV_{Ple} C the importance of father-offspring relationships and male immigration may have masked any effect of contrasting levels of between-pride competition. Unlike the other subtypes, FIV_{Ple} A was relatively rare and much less genetically diverse compared to subtypes B and C, as it was represented by only one OTU. The reduced diversity of subtype A may result from reduced transmission efficiency or a more recent introduction into the Serengeti (Troyer *et al.* 2011). One caveat is that low abundance viral sequences may be missed by PCR amplification (Troyer *et al.* 2011).

Mother-offspring relationships were important for FIV_{Ple} C and B which suggests that vertical transmission is likely to play a role for transmission of FIV_{Ple} in lions, as has been demonstrated for solitary cats species like puma (Carpenter *et al.* 1996; Biek *et al.* 2003). It was hypothesized that vertical transmission may be more important for subtype B, as FIV_{Ple} C prevalence increases in 1-2 year old lions (Troyer *et al.* 2011), yet this observation may just be a result of sparse sampling from lions less than one year. Our finding that vertical transmission is likely to be important, combined with serological evidence that FIV_{Ple} can be transmitted horizontally in lions (Brown *et al.* 1994), indicates that mixed-mode transmission likely underlies the dynamics for B and C subtypes of FIV_{Ple}. Mixed mode transmission was considered likely for FIV transmission in a North American puma population (Biek *et al.* 2003), for Simian Foamy Virus (SFV) in chimpanzees (*Pan troglodytes*) (Blasse *et al.* 2013), and may have widespread importance in wildlife/pathogen systems (Ebert 2013).

The absence of a pre/post-CDV effect on FIV_{Ple} C transmission dynamics may be due to the importance of male transmission at both within- and between-pride scales. The FIV_{Ple} C pride-

level effect largely results from vertical transmission from both father and mother, whereas subtype-B transmission primarily results from mother to offspring combined with within-pride horizontal transmission. The post-CDV reduction in lion density would be unlikely to affect the paternally transmitted FIV_{Ple} C, as male coalition size and immigration events remained similar across both time periods (Tables S4 and S5. However, these results were based on a relatively small subset of the data with known parent-offspring relationships. Further host genomic analysis would be required to resolve the within-pride relatedness effects on FIV_{Ple} transmission.

Our results provide one of the first examples of biparental transmission of a pathogen in social mammals. Although evolutionary theory predicts that biparental transmission may increase virulence and diversity of the pathogen compared to those that are maternally transmitted (Ebert 2013), subtype C was not as diverse as subtype B, which may indicate that another transmission mechanism, selective pressure, or earlier introduction of FIV_{Ple} C into the population (Troyer *et al.* 2011) may be responsible for the lower diversity. As males are the primary dispersers, another consequence of male transmission of FIV_{Ple} C is that pathogens may be directly transmitted beyond their local neighbourhood, epidemiologically connecting larger lion populations: nomadic males may therefore play a larger role in FIV_{Ple} C transmission than for pathogens with shorter infectious periods such as CDV (Craft *et al.* 2011).

The differences in within-pride transmission dynamics for FIV_{Ple} B and C manifest in the observed between-pride transmission patterns. Of the spatial and social networks, distance between prides was an important of between-pride transmission networks for FIV_{Ple} B, highlighting the importance of localized transmission events between neighbouring prides. In contrast, male immigration was an important predictor of the FIV_{Ple} C transmission network, indicating that long distance transmission events by dispersing males is a critical transmission

pathway, however distance between prides played a secondary role in the transmission of FIV_{Ple} C. Thus, FIV_{Ple} transmission is a composite of localized and long distance contacts mediated by effects of FIV_{Ple} co-infection patterns. Intriguingly, our between-pride results suggest that for FIV_{Ple} transmission the types of contact required could be subtype dependent Aggressive and familial contacts may be more important for FIV_{Ple} B, whereas sexual contact may be more likely to transmit FIV_{Ple} C. Similar patterns have been found in HIV 1 and HIV 2 (De Cock *et al.* 1993; e.g., Hu *et al.* 1999), where HIV 2 has a much lower rate of sexual transmission compared to HIV 1 (De Cock *et al.* 1993)

One caveat with our findings is that the social and spatial networks are dynamic; important variation in network structure at a finer scale could have been important for some transmission events, but could be lost when the data was averaged. For example, hypothetically two prides could have shared males for three years (out of 33) and this could have led to shared OTUs between prides, but because we averaged the shared male network over time this interaction would have been down-weighted. Whilst we cannot rule out that this occurred, the impact likely to be limited as this situation was rare in our data. Finer temporal resolution of each spatial or social network was statistically unfeasible due to the sparseness of the data.

Our between-pride results suggest that for FIV_{Ple} transmission the types of contact required are different between subtypes and the role of other FIV_{Ple} infections could be subtype dependent. We have demonstrated that the distribution of subtypes and specific OTUs are linked, but the mechanisms behind these patterns are unclear. One potential mechanism for this is OTU specific co-operation between strains to maintain fitness (see Turner & Chao 1999 for a potential physiological pathways). For example, the positive association between FIV_{Ple} A1 and B1 (Fig. S8) may be indicative that OTUs can act synergistically in co-infections. This type of synergism

has been demonstrated within a host for strains of HIV (e.g., Wang *et al.* 2000), and may also underlie FIV_{Ple} infection more broadly. However, there may also be some competition between strains as prides with high FIV_{Ple} B prevalence (mostly consisting of B1) were less likely to share FIV_{Ple} C (Fig. S8). Competition between HIV strains have also been demonstrated in *in-vitro* experiments (e.g., Quiñones-Mateu *et al.* 2000). Within the large amount of diversity present in retroviruses, it seems likely that different subtypes, strains, or OTUs may have differing coexistence strategies. Regardless of the co-occurrence mechanism, our results indicate that co-infection patterns can have population-level consequences on transmission. It is also possible that each subtype may be transmitted the same way, but within-host pathogen interactions alter the infection dynamics and fitness of each pathogen, and therefore the sequence we sampled from each individual could be biased. All of these associations found in this wild population may serve to generate hypotheses that could be followed up with experimental contests. Our study highlights the importance of monitoring a wild animal population over time in order to generate such insights, given that these patterns may be difficult to untangle in controlled experimental contexts.

We found that FIV_{Ple} subtypes have different transmission pathways at all scales, and, as with HIV, FIV should not be considered as one pathogen epidemiologically: each FIV_{Ple} subtype had distinctive transmission pathways. These results have important implications for understanding FIV ecology and landscape-level disease management. Similar approaches could be employed to transmission studies of retroviruses such as simian immunodeficiency virus (SIV) or feline foamy virus (FFV) to determine whether subtype differences are also important in those systems. Viral phylodynamics coupled with community phylogenetic ecology techniques can infer transmission dynamics over multiple scales, and thereby reveal insights into the differential

evolutionary pressures acting on virus subtypes and how these can manifest into landscape level effects.

Statement of authorship: NFJ performed the analyses and wrote the first draft of the manuscript. MC and JT conceived the project. MJ, KV, CP, MJ, and SR helped with analyses and collated data. All authors contributed substantially to revisions of the paper.

Data accessibility

Sequences used in this study are available in GenBank (GenBank accession numbers AY549217-AY549304, AY552614 to AY552683, and AY552684 - AY552748, and AY878208 - AY878235). All other data are accessible from the Dryad Digital Repository http://dx.doi.org/10.5061/dryad.8j3s3 (Fountain-Jones et al. 2017).

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