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Research Article

Habitat connectivity and host relatedness influence virus spread across an urbanising landscape in a fragmentation-sensitive carnivore

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Abstract

Spatially heterogeneous landscape factors such as urbanisation can have substantial effects on the severity and spread of wildlife diseases. However, research linking patterns of pathogen transmission to landscape features remains rare. Using a combination of phylogeographic and machine learning approaches, we tested the influence of landscape and host factors on feline immunodeficiency virus (FIV_{Lru}) genetic variation and spread among bobcats (*Lynx rufus*) sampled from coastal southern California. We found evidence for increased rates of FIV_{Lru} lineage spread through areas of higher vegetation density. Furthermore, single-nucleotide polymorphism (SNP) variation among FIV_{Lru} sequences was associated with host genetic distances and geographic location, with FIV_{Lru} genetic discontinuities precisely correlating with known urban barriers to host dispersal. An effect of forest land cover on FIV_{Lru} SNP variation was likely attributable to host population structure and differences in forest land cover between different populations. Taken together, these results suggest that the spread of FIV_{Lru} is constrained by large-scale urban barriers to host movement. Although urbanisation at fine spatial scales did not appear to directly influence virus transmission or spread, we found evidence that viruses transmit and spread more quickly through areas containing higher proportions of natural habitat. These multiple lines of evidence demonstrate how urbanisation can change patterns of contact-dependent pathogen transmission and provide insights into how continued urban development may influence the incidence and management of wildlife disease.

Key words: Viruses; transmission; urbanisation; disease; landscape; phylogenetics; bobcat.

Introduction

Understanding how host and landscape factors influence pathogen transmission is a primary focus of epidemiology and disease ecology (Gottdenker et al. 2014; Becker, Streicker, and Altizer 2015). Because natural and anthropogenic landscape features are spatially heterogeneous, the distribution of pathogen transmission (i.e. pathogen transmission networks) and spread of disease outbreaks are similarly heterogeneous (Ostfeld, Glass, and Keesing 2005; Meentemeyer, Haas, and Václavík 2012). However, relating pathogen transmission to specific landscape factors has been challenging because observing transmission events in wild populations is difficult. Inference of contacts among hosts (often by direct observations or measuring spatial overlap) has been a valuable proxy for transmission but often requires intensive effort and may not accurately reflect transmission (Craft 2015; Gilbertson, Fountain-Jones, and Craft 2018). Advances in molecular data collection and analysis are enabling researchers to quantify transmission more precisely (Archie et al. 2009; Biek and Real 2010; Didelot et al. 2017; Kozakiewicz et al. 2018). These advances improve our ability to elucidate the impacts of landscape features on pathogen dynamics, which remain poorly understood.

Viruses are excellent candidates for the genetic inference of pathogen transmission. Their relatively small genomes and rapid evolutionary rates produce generally high degrees of genetic

variation across transmission networks over ecologically relevant timescales, recording where and when pathogen outbreaks have occurred and enabling identification of transmission among extant populations or even individual hosts (Archie et al. 2009; Brunker et al. 2012; Biek et al. 2015; Fountain-Jones et al. 2022). Consequently, genetic approaches have increased our understanding of transmission in many important pathogens spreading through host populations, including Middle East respiratory syndrome coronavirus (MERS-CoV; e.g. Corman et al. 2014), Ebola (e.g. Carroll et al. 2015), influenza (e.g. Magee et al. 2015), human immunodeficiency virus (HIV; e.g. Faria et al. 2014), rabies (e.g. Streicker et al. 2016), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV2; e.g. Forster et al. 2020; Li et al. 2021). In addition to viruses causing overt disease, agents that spread within a host species as a chronic infection resulting in little pathogenicity enable the study of transmission networks at any time post-infection. Viruses infecting blood cells or shed via body fluids, enabling pre-mortem and repeated sampling, also favour analysis of transmission. When such agents are sufficiently prevalent within populations, it is feasible to obtain sample sizes that facilitate the analysis of factors influencing transmission. Accordingly, sophisticated molecular tools have been developed to quantify how specific heterogeneous landscape factors shape viral transmission at fine spatiotemporal scales. For example, 'ecophylogenetic' approaches integrating phylodynamics (the study of processes shaping viral phylogenies; Grenfell et al. 2004), landscape ecology, and community ecology can reveal how interactions between host and pathogen communities, as well as environmental factors, shape pathogen dynamics (Dellicour et al. 2016; Fountain-Jones et al. 2017c). Similarly, emerging machine learning approaches provide a powerful means of disentangling the complex relationships between genetic variation and environmental processes, bringing fresh insights into pathogen ecology and evolution (Fountain-Jones, Smith, and Austerlitz 2021).

Feline immunodeficiency virus (FIV) is a rapidly evolving and largely species-specific RNA retrovirus that infects many wild felids (VandeWoude and Apetrei 2006; Carver et al. 2016). FIV integrates a DNA copy of its genome within the infected host that persists for the lifetime of the animal, and thus, phylogenetic analysis of this agent has provided insights into pathogen dynamics as well as host population dynamics in several species (Biek, Drummond, and Poss 2006; Lee et al. 2012; Fountain-Jones et al. 2017a,b; Kozakiewicz et al. 2020). FIV is endemic in many bobcat (*Lynx rufus*) populations (species-specific strain; FIV_{Lru}) at a relatively high prevalence (up to 25 per cent; Carver et al. 2016; Kozakiewicz et al. 2020) and is thus well suited to inferring the impacts of host and landscape factors on pathogen transmission. Furthermore, bobcats are useful indicators of functional landscape connectivity in fragmented urban systems (Crooks 2002; Kozakiewicz et al. 2019), particularly in coastal southern California, where major highways and dense tracts of urban development form significant barriers to connectivity among bobcat populations (Riley et al. 2006; Lee et al. 2012; Ruell et al. 2012; Serieys et al. 2015; Kozakiewicz et al. 2019; Smith et al. 2020). FIV_{Lru} in these populations has therefore been extensively used as a model for investigating virus transmission in wildlife, and our understanding of this system has evolved significantly over time as sampling continued and data analyses have become increasingly sophisticated. Previous studies of FIV_{Lru} transmission in these populations have either focused on a relatively small geographic area (Lee et al. 2012; Fountain-Jones et al. 2017a; Fountain-Jones et al. 2021) or have encompassed the entire region but focused only on a broad-scale phylogeographic structure with respect to major barriers separating host populations (Kozakiewicz et al. 2020). Given that FIV_{Lru} is transmitted via direct contact between hosts (VandeWoude and Apetrei 2006), genetic co-structuring is expected between virus and host. Yet, evidence for this is mixed, suggesting that the extent to which co-structuring occurs may be dependent on the environmental context (Lee et al. 2012; Kozakiewicz et al. 2020). Thus, there is a need to quantify and compare environmental and host factors influencing FIV_{Lru} across multiple populations and spatial scales.

Here, we tested whether host and landscape factors influence virus transmission and spread, using FIV_{Lru} in coastal southern California as a model. Building on previous work in this region (see Lee et al. 2012; Fountain-Jones et al. 2017a; Fountain-Jones et al. 2021), we aimed to elucidate how factors influencing pathogen transmission and spread can differ with spatial scales (local and regional) and among bobcat populations experiencing different urban impacts. We focused on two components of FIV_{Lru} transmission. First, we quantified the rates and routes of pathogen spread and identified influential landscape factors measured along these routes. Second, using FIV_{Lru} SNP variation as a proxy, we assessed how transmission frequency is influenced by host factors (sex and genetic variation) and variations in urban and natural landscape factors measured at sample locations. Given that bobcats are habitat generalists and that urbanisation is the predominant factor driving connectivity among host populations (e.g. Kozakiewicz et al. 2019), we expected that rates of virus spread would be reduced through urban areas, but that certain landscape features (e.g. streams) may enhance virus spread through these areas. Because FIV_{Lru} transmission relies on direct host contact, we also expected that FIV_{Lru} SNP variation would be associated with host genetic variation but that this association might also be influenced by variation in local natural and urban landscape factors. Ultimately, these complementary approaches provided detailed insights into how landscape factors shape spatiotemporal patterns of virus transmission in free-living wildlife in one of the world's most urbanised regions.

Methods

FIV_{Lru} samples and sequence analysis

We used FIV_{Lru} sequences previously collected as part of a large screening effort that included samples from 292 bobcats from three adjoining areas in coastal southern California (Fig. 1). Original blood and tissue samples were collected as part of earlier studies as follows: forty-five were collected from the north and east of San Diego between 2007 and 2012 (Jennings and Lewison 2013), 113 from the southeast of Los Angeles (LA) between 2002 and 2010 (Lyren et al. 2006, 2008a,b), and 134 from the northwest of LA between 1996 and 2011 (Riley et al. 2006; Serieys et al. 2015). Collectively, these samples represent five genetically distinct populations separated by major highways and urban areas (Kozakiewicz et al. 2019).

DNA was extracted and samples were screened and sequenced for FIV_{Lru} previously by Kozakiewicz et al. (2020). A subset of sequences was obtained from Lee et al. (2014); original publications and Genbank accession numbers for each sequence are indicated in Supplementary Material 2. We trimmed all sequences to include only the *env* open reading frame (ORF), excluding stop codons, for a final length of 1,257 bp. The final alignment included fifty-one FIV_{Lru} sequences. We examined aligned ORFs for recombination breakpoints using Recombination Detection Program (RDP) v4.96 (Martin et al. 2015) with several recombination detection methods: RDP (Martin and Rybicki 2000), GENECONV (Padidam, Sawyer, and Fauquet 1999), Chimaera (Posada and Crandall 2001), MaxChi (Maynard Smith 1992),

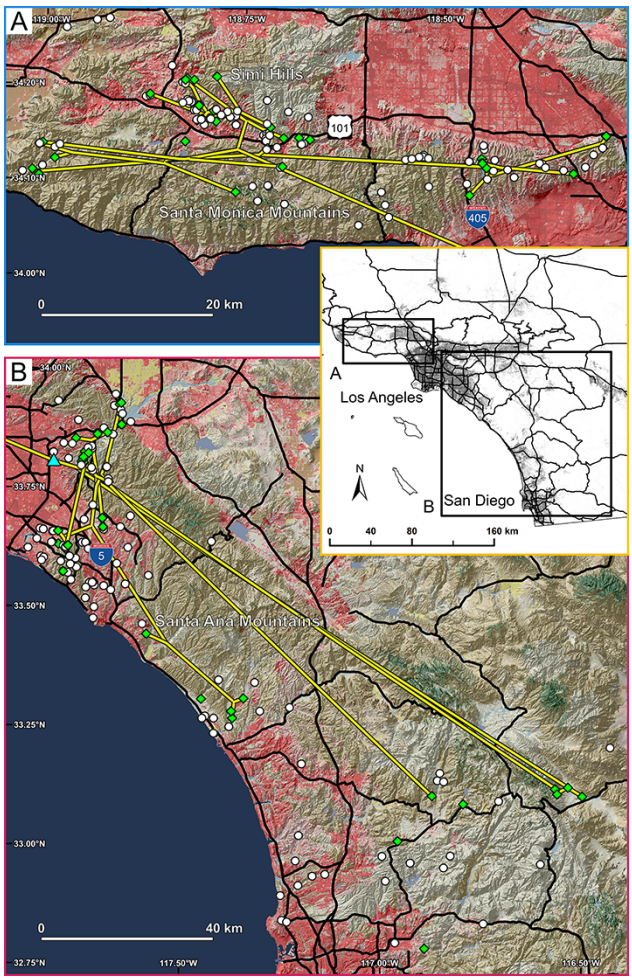

Figure 1. A continuous Bayesian phylogeographic analysis depicts the spatiotemporal dispersal of FIV_{Lru} in bobcats. Yellow lines indicate bifurcating branches showing phylogenetic relationships, originating from an ancestral node shown as a light blue triangle. Circles and diamonds indicate bobcat sample locations, with FIV_{Lru}-positive samples shown as green diamonds and FIV_{Lru}-negative samples shown as white circles. Areas northwest (A) and southeast (B) of LA are shown, with a single branch joining the two areas. The base map colours indicate land cover according to the default NLCD colour scheme, with urbanisation indicated as varying shades of red (darker red indicates higher density urban land cover, whereas pink/grey indicates lower density urban land cover), shrub/scrub indicated as tan, forests indicated as green, and agriculture indicated as yellow. Elevation variation is depicted as shaded relief. The black-and-white inset (reproduced from Kozakiewicz et al. 2020) indicates the extent of the region-wide study area, with cities of LA and San Diego shown and shaded areas indicating urban land cover.

BootScan (Salminen et al. 1995), SiScan (Gibbs, Armstrong, and Gibbs 2000), and 3Seq (Boni, Posada, and Feldman 2007). Recombination breakpoints were accepted if they were detected using at least three of these methods at a significance level of $P < 0.05$. Any recombinant regions were removed from subsequent analyses.

Phylogenetic analysis

To ensure sequence data contained sufficient temporal signal for the estimation of divergence dates, we performed date randomisation tests using the R package *TipDatingBeast* (Rieux and Khatchikian 2017). Sequences potentially biasing temporal inferences were removed based on leave-one-out cross validation (Duchêne et al. 2015). To reconstruct the spread of FIV_{Lru} lineages across the coastal southern Californian landscape, a Bayesian phylogeographic analysis of viral diffusion in continuous space was conducted using Bayesian Evolutionary Analysis Sampling Trees (BEAST) version 1.10 (Drummond et al. 2012). Tree tip dates were specified according to the sample date, with sampling location (latitude and longitude) specified as a continuous trait.

Prior to incorporating phylogeographic random walk models, which implement the spatial component of phylogenetic diffusion, we performed a series of BEAST runs testing different substitution models and molecular clocks and selected the most appropriate using marginal likelihood estimation with path and stepping-stone sampling (Baele et al. 2012). The best-supported models included the Hasegawa-Kishino-Yano substitution model with gamma-distributed rate heterogeneity and a proportion of invariant sites, the two-partition codon partition model, the Gaussian Markov random field Bayesian Skyride tree prior, and a lognormal uncorrelated relaxed molecular clock (Kozakiewicz et al. 2020). Using these parameters, relaxed random walk (RRW) models were tested against a model assuming no dispersal rate variation among branches (Brownian random walk). The gamma RRW model was chosen, having significantly higher support than the lognormal RRW and Brownian models and marginally higher support than the Cauchy RRW (see Supplementary Material 1, Table S1, for all model selection results). We ran three sets of 10^8 Markov chain Monte Carlo iterations, sampling every 10^4 iterations and excluding the initial 10 per cent of each set as burn-in. Model stationarity, convergence, and effective sample size (minimum 200) were checked, and parameters were evaluated using Tracer version 1.7 (Rambaut et al. 2018). A maximum clade credibility tree was constructed from the sampled trees using TreeAnnotator version 1.10 and visualised using FigTree version 1.4.3. To visualise the spatiotemporal distribution of viral lineages based on the maximum clade credibility tree, we used SPREAD3 (Bielejec et al. 2016) (Fig. 1).

Analysis of FIV_{Lru} spatial spread

We quantified rates of FIV_{Lru} spread using *Seraphim* (Dellicour et al. 2016) in R. One thousand trees were randomly sampled from our BEAST posterior distribution, and from each tree, we extracted dates and locations associated with the start and end of each branch. Using this spatiotemporal information, we estimated dispersal statistics that describe the median velocity of FIV_{Lru} dispersal (in km/y) and the mean weighted diffusion coefficient (defined as diffusivity or the rate of spread in km^2/y; Trovão et al. 2015). In contrast to dispersal velocity, which measures the linear dispersal rates of individual phylogenetic branches, the weighted diffusion coefficient is a cumulative estimate of branch dispersal velocities that measures the rate of expansion of the area into which viral lineages have spread or 'diffused'.

We tested the effects of five heterogeneous landscape factors on dispersal velocities associated with phylogenetic branches using a landscape connectivity framework. Landscape variables were chosen based on predicted importance to bobcat movement ecology. Topographic roughness, vegetation density, and streams were predicted to have positive effects on bobcat (and thus FIV_{Lru}) connectivity, while urbanisation (measured as a percentage of impervious surface) and roads were predicted to have negative effects. An ecological rationale for the inclusion of landscape variables and data sources are provided by Kozakiewicz et al. (2019). Parameterisation of resistance surfaces (i.e. translation of environmental measurements to values of potential resistance to movement) was performed using an optimisation approach

implemented in *ResistanceGA*, which uses a genetic algorithm to explore various resistance surface parameterisations to maximise the correlation between genetic distances and resistance surface cost values (Peterman 2018). Optimisation approaches are considered preferable to alternatives such as expert opinion, which can be subject to experimenter bias, or habitat suitability models, which do not necessarily reflect ease of movement (Spear et al. 2010; Elliot et al. 2014). Pairwise genetic distances between individual bobcats were used for resistance surface optimisation, which was performed singly for each landscape variable based on random-walk commute time between locations (McRae 2006; McRae et al. 2008). Bobcat genetic distance was measured as the inverse of the proportion of shared alleles across 13,520 SNP loci generated using double-digest restriction-site-associated DNA sequencing (see Kozakiewicz et al. 2019 for details). Successive generations of the genetic algorithm were tested using linear mixed effect models with maximum likelihood population effects and evaluated using log-likelihood scores. Optimal parameterisation was determined following twenty-five consecutive generations of the genetic algorithm with no improvement in log-likelihood. The effect of geographic distance was included in all models.

Using *Seraphim*, we compared two connectivity approaches for estimating environmental distances among FIV_{Lru} phylogenetic branch start and end locations for each resistance surface: (1) least-cost path analysis (Adriaensen et al. 2003), which models a single, optimal path between locations; and (2) resistance analysis, implemented using Circuitscape (McRae et al. 2008; Hall et al. 2021), which models all possible paths between locations. Branch durations were linearly regressed against associated least-cost paths and resistance distances for each landscape factor while accounting for the effect of geographic distance alone (a null model) to obtain a regression coefficient, R, and a modified coefficient of determination, Q. The effect of environmental distance on branch duration is positive when $R > 0$ and is greater than that of the null model when $Q > 0$. Values for R and Q were calculated for each of the 1,000 sampled trees to obtain distributions of each. Landscape factors were considered potentially explanatory when the proportion ($Q > 0$) was greater than 0.9 and when 95 per cent confidence intervals for R were greater than zero. The significance of Q distributions for potentially explanatory landscape factors was estimated using Bayes factor support calculated by randomisation of branch locations for each sampled tree and interpreted according to Kass and Rafferty (1995). Only branches with start dates after 1980 were included in tests of landscape effects to reduce the temporal mismatch between estimated dispersal velocities and present-day landscape factors. We selected a cut-off date of 1980 as it was an appropriate compromise between removing the most temporally mismatched branches without removing so many branches as to significantly reduce our statistical power to detect correlations with landscape factors.

Previous analysis estimated that FIV_{Lru} populations northwest of LA diverged from those southeast of LA c. 1875 (Kozakiewicz et al. 2020). Furthermore, significant differences in landscape composition, as well as bobcat population structure, have been observed between these two areas (Riley et al. 2010; Kozakiewicz et al. 2019). To evaluate potential differences among areas in rates and landscape drivers of FIV_{Lru} spatial spread, analyses of FIV_{Lru} spread were performed separately for three distinct study areas: (1) northwest of LA, (2) southeast of LA, and (3) across the entire region comprising all sampled individuals (hereafter referred to as the 'region-wide' study area) (Fig. 1).

Landscape and host effects on FIV_{Lru} genetic variation

To test how FIV_{Lru} genetic variation is associated with the host (sex and genetic relatedness/distance) and landscape factors, we constructed multivariate machine learning models using Multi-response Interpretable Machine Learning (MrIML; Fountain-Jones et al. 2021). MrIML is a flexible pipeline for producing, comparing, and interpreting machine learning models for predicting drivers of variation in multilocus datasets. First, we extracted single SNP genotypes from FIV_{Lru} multiple sequence alignments using SNP-sites (Page et al. 2016). SNPs were filtered to only include biallelic loci using VCFtools (Danecek et al. 2011) so that they could be analysed using MrIML's classification framework. A small number of SNP genotypes were missing for some isolates; these were set as the major allele for a given locus. SNPs with allele frequency <0.2 were also removed because the models implemented in MrIML have limited power to predict rare response conditions.

Genetic differentiation among viruses present in different environments may reflect variation in host dispersal preferences or local adaptation by either pathogens or hosts, leading to differences in environmentally mediated transmission frequency. Thus, we used our machine learning models to test how differences in landscape composition in the immediate vicinity of sample locations influence FIV_{Lru} genetic variation. Landscape factors comprised a series of land cover types derived from the National Land Cover Database (NLCD; Homer et al. 2015) and were quantified within buffers around sample locations. Buffers were based on estimates of sex and area-specific mean home range sizes (northwest LA: males = 7.1 km^2, females = 3.5 km^2; southeast LA: males = 6.8 km^2, females = 4.4 km^2; San Diego: males = 5.3 km^2, females = 3.8 km^2; Riley et al. 2010). The relative proportions of each urban (broken into separate categories comprising developed open space and low, medium, and high-density urbanisation) and non-urban (separate categories comprising forest, scrub/shrub, and grassland) land cover type were quantified within each buffer using Geospatial Modelling Environment (www.spatialecology.com). We also calculated the distance of each capture location to the nearest urban edge using the Near tool in ArcGIS 10.3 (ESRI). Urban edge was defined from the NLCD impervious surface layer by calculating for each 30 × 30 m raster cell whether most cells within a 1 km radius of that cell had a value of impervious surface greater than zero.

Host genetic distances were measured as described earlier. Because MrIML requires each predictor to be a single vector, we performed principal coordinate analysis to reduce the dimensionality of the pairwise host genetic distance matrix. We used the top nine principal coordinates as predictor variables in our analysis (cumulatively explaining 38.4 per cent of variation in host genetic distances; each remaining principal coordinate explained <2 per cent). Each principal coordinate captures a different component of host genetic variation, with the first principal coordinate capturing the broadest-scale patterns of variation and each successive principal coordinate capturing increasingly fine patterns. All landscape and host variables were included as predictors of FIV_{Lru} SNP variation. To account for potential temporal autocorrelation (i.e. increased similarity between sequences sampled at similar times), sample date was included as an additional predictor. Latitude and longitude were included to account for spatial autocorrelation.

Using MrIML, we tested three types of classification models to predict FIV_{Lru} SNP responses to landscape and host factors (in order of increasing model complexity): generalised linear models (GLM), random forests (RF), and extreme gradient boosting (XGB).

RF and XGB models comprised 1,000 trees, and for all models, we used MrIML's inbuilt parameter tuning function with a tuning grid size of 5 × 5 to automatically select optimal model parameter combinations. For each SNP in a given model, MrIML builds a separate binary classifier and calculates the area under the curve of the receiver operating characteristic (AUC-ROC), which measures the ability of the model to accurately distinguish between response classes (i.e. allele values) and is given as a proportion ranging between 0 (no predictive ability) and 1 (perfect predictive ability). Overall model performance was evaluated by averaging AUC-ROC across all SNPs. Model-agnostic variable importance (VI) was estimated from the best-performing model for each FIV_{Lru} SNP individually as well as cumulatively across all SNPs. To visualise average SNP responses to variation in the most important predictors, we produced accumulated local effects (ALE) plots using MrIML. ALE plots are capable of distinguishing effects among correlated variables, which makes them suitable for datasets containing proportional variables (such as land cover), which are inherently intercorrelated (Supplementary Material 1, Tables S2–S4). Finally, to evaluate interactions among host and landscape factors in influencing FIV_{Lru} SNP variation, we used MrIML to produce plots showing relative importance for all pairwise combinations of explanatory variables, cumulatively across all SNPs. As with the investigation of landscape drivers of FIV_{Lru} spread, analyses were performed separately for the northwest of LA, the southeast of LA, and region-wide study areas.

Results

Sequence analysis

Among our alignment of fifty-one FIV_{Lru} sequences, we identified no regions exhibiting signatures of recombination. One sequence was found to bias our temporal estimates and was removed. Thus, fifty sequences remained for further analysis, comprising twenty-six from the northwest of LA and twenty-four from the southeast of LA.

FIV_{Lru} spatial spread is weakly influenced by landscape

Dispersal velocities are the linear rate at which individual phylogenetic branches move across the landscape, whereas the weighted diffusion coefficient is the rate of expansion of the area occupied by the phylogeny. We found minimal differences in both metrics between each of our focal study areas, and neither study area exhibited differences in these metrics on a region-wide scale. However, weighted diffusion coefficients were more highly variable southeast of LA (Fig. 2). These results suggest minimal differences in the rate of FIV lineage spread on the landscape between northwest and southeast LA.

For both least-cost paths and landscape resistance modelling, there was low support for landscape factors affecting FIV_{Lru} dispersal velocities. For least-cost path analysis, region-wide vegetation density had marginally greater explanatory power than the null model [proportion $(Q > 0) = 0.70$] but had substantial Bayes factor support ($BF = 4.8$). This relatively low proportion $(Q > 0)$ suggests a weak positive effect of vegetation density on dispersal velocity that is not present in all the sampled phylogeographic trees but is significantly based on Bayes factor estimation. Otherwise, only streams in the southeast of LA had (marginally) greater explanatory power [proportion $(Q > 0) = 0.83$] than the null model, but this effect had little Bayes factor support ($BF = 1.8$). Using landscape resistance modelling, the best, albeit poorly, supported association was streamed southeast of LA [proportion $(Q > 0) = 0.50$, $BF = 4.2$]; no association greater than [proportion $(Q > 0) = 0.21$] was observed for any other landscape factor within any of the study areas under the landscape resistance modelling framework. See Supplementary Material 1, Table S5, for full Seraphim results.

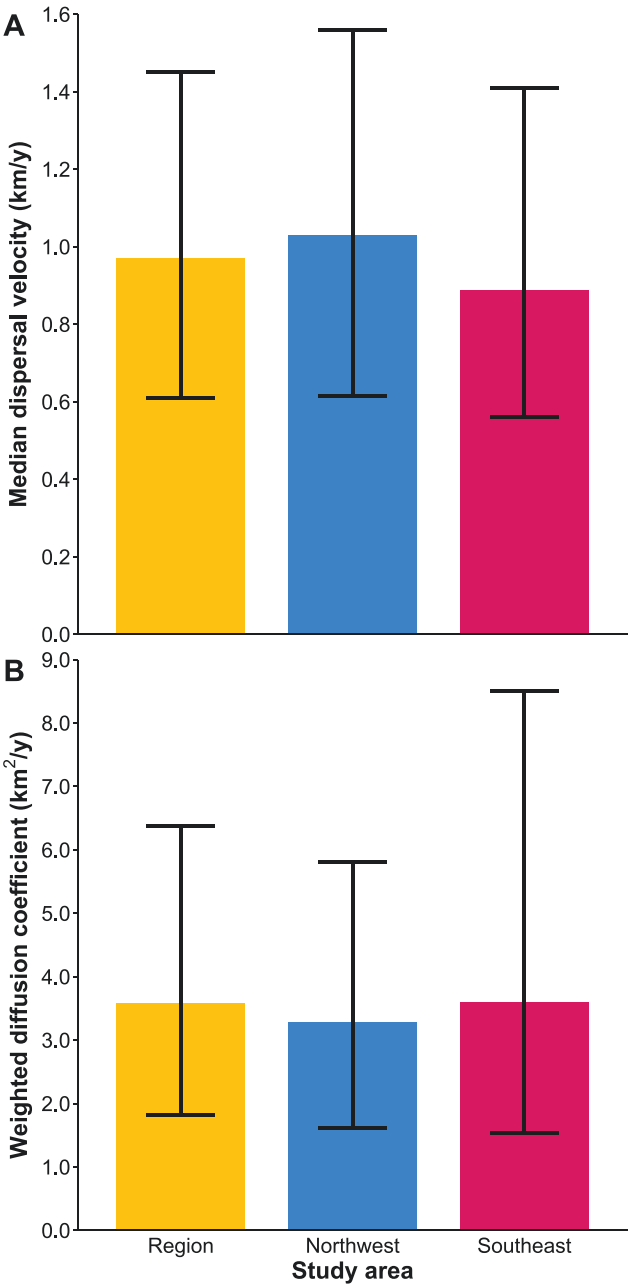

Figure 2. FIV_{Lru} phylogenetic branch dispersal velocities (A) and diffusion rates (B) do not differ significantly between the northwest and southeast of LA local study areas or between local study areas and the region-wide scale. The error bars indicate 95 per cent highest posterior density intervals.

FIV_{Lru} SNP variation is driven by natural land cover and co-structures with hosts

Region-wide, the best-performing model of host and landscape factors explaining FIV_{Lru} SNP variation was the RF, with an AUC-ROC of 0.852, suggesting that the model had a high capacity to predict SNP alleles. In contrast, GLM and XGB had AUC-ROC of

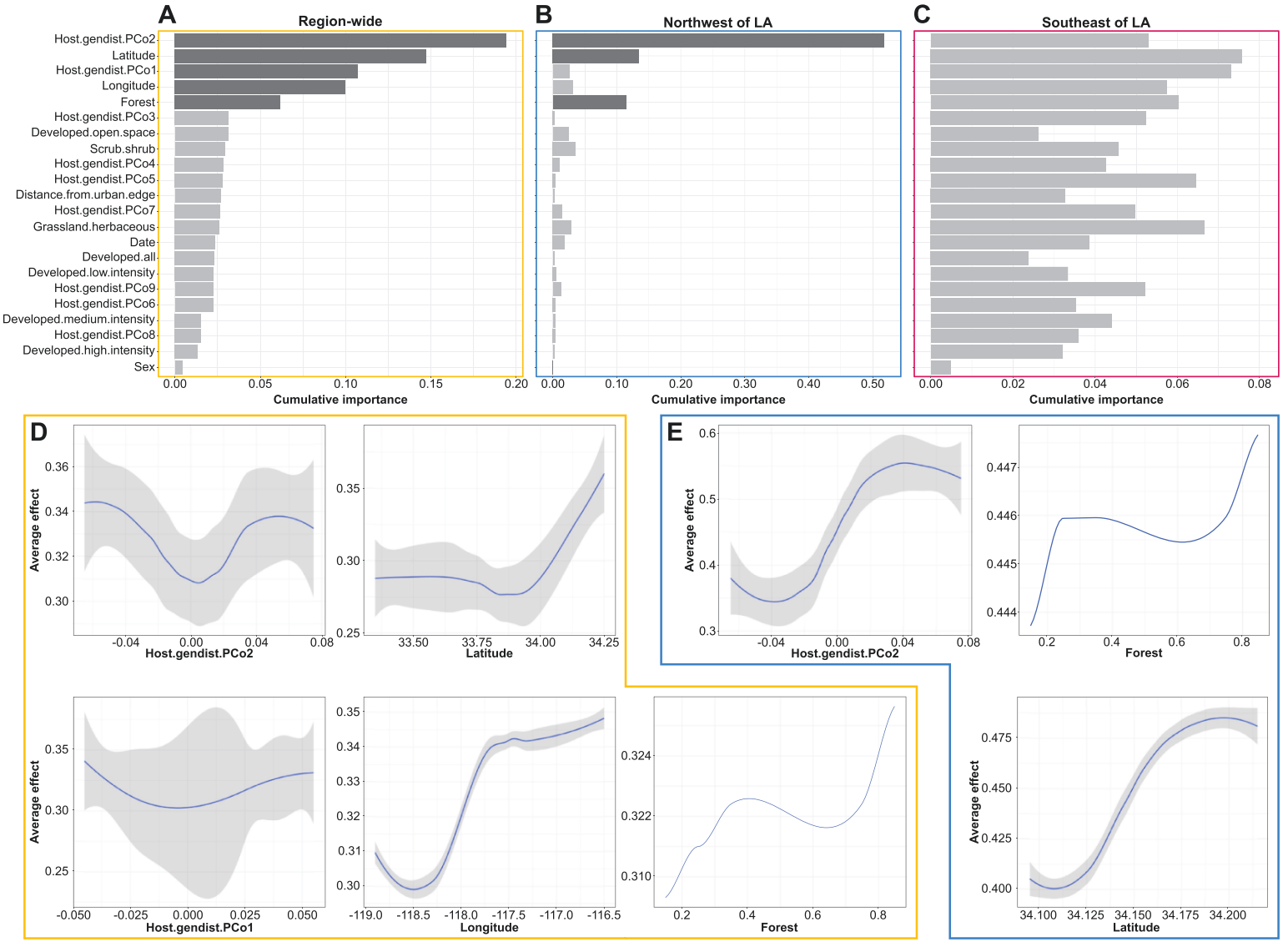

Figure 3. RF model of FIV_{Lru} SNP variation. (A–C) The relative importance of host and landscape factors in explaining cumulative FIV_{Lru} SNP variation region wide (A), the northwest of LA (B), and the southeast of LA (C). Y-axis labels are shared across plots and are in order of variable importance from panel A. Dark grey bars indicate variables of high relative importance, whereas light grey bars indicate variables of low relative importance. Note that X-axis scale differs between plots. (D, E) ALE plots showing the effects of important predictors of FIV_{Lru} SNP variation region wide (D) and northwest of LA (E), with grey shading indicating 95 per cent confidence intervals. For ALE plots, variation along Y-axes (average effect) reflects relative increases and decreases in the probability of the presence of the minor allele at a given feature (X-axis) value. Host.gendist.PCo1 primarily captures genetic differentiation between bobcats northwest and southeast of LA, whereas Host.gendist.PCo2 primarily captures bobcat genetic variation within the area northwest of LA. PCo values represent the position along each axis of variation, with higher and lower values reflecting greater distance from the mean (zero). Note that the ALE plots for Forest lack confidence intervals due to the low number of SNPs contributing to these cumulative patterns. ALE plots for southeast of LA are not shown because no variables were of high relative importance.

0.757 and 0.500. The improvement in the predictive performance of RF over GLM indicates the importance of non-linear relationships in our dataset. Two principal coordinates representing host genetic distances (PCo 1 and 2, VI = 0.107 and 0.194, respectively), as well as latitude (VI = 0.147) and longitude (VI = 0.100), were the most important predictors of overall FIV_{Lru} SNP variation (Fig. 3A). Forest land cover was also of prominent importance (VI = 0.062). All other factors tested had lower and approximately equivalent VI (~0.01 to 0.025) and were not considered further (Fig. 3A).

ALE plots revealed that the steepest gradient of FIV_{Lru} SNP variation region-wide coincided with the strong genetic barrier formed by LA itself, occurring between approximately 118.25–117.75° W and 33.90–34.25° N (Fig. 3D). Host genetic distance PCo1 primarily represented the genetic differentiation between bobcats sampled northwest and southeast of LA, explaining 6.4 per cent of total host genetic variation. PCo2, in contrast, primarily captured bobcat genetic variation northwest of LA, explaining 4.8 per cent of total host genetic variation (Supplementary Material 1, Fig. S1). Despite explaining a lower proportion of host genetic variation, PCo2 was the more important predictor of FIV_{Lru} SNP variation (Fig. 3A), with ALE plots revealing a sharp distinction between FIV_{Lru} isolates sampled from even slightly genetically divergent hosts (Fig. 3D). PCo1, in contrast, revealed differentiation between FIV_{Lru} isolates only when hosts were highly genetically divergent (Fig. 3D). FIV_{Lru} sequences collected from locations with a high proportion (>0.8) of forest land cover were distinct from those collected from locations with low or no forest land cover (Fig. 3D).

A high degree of interaction was observed among many of our explanatory variables. However, only a proportion of interactions were of prominent relative importance (interaction relative importance >0.25, cumulative across all SNPs). Of these, the most important interaction was between host genetic distance PCo2 and grassland land cover, with a cumulative relative variable importance of 0.392.

When analysing FIV_{Lru} sequences collected from northwest of LA, the RF model again performed best, with an AUC-ROC of 0.897. Host genetic distance PCo2 was by far the most important predictor of FIV_{Lru} SNP variation (VI = 0.518), followed by latitude (VI = 0.135) and forest land cover (VI = 0.115) (Fig. 3B). ALE plots revealed similar patterns to the region-wide analysis, with relatively low genetic differentiation between hosts resulting in high differentiation between FIV_{Lru} isolates (Fig. 3E). There was a dramatic shift in FIV_{Lru} SNP variation between the latitudes of 34.125–34.175° N (Fig. 3E), which is approximately the latitude at which Highway 101 crosses the study area east-to-west (Fig. 1).

When analysing FIV_{Lru} sequences collected from the southeast of LA, we observed overall lower model performance, with the RF model performing best (AUC-ROC = 0.703). Accordingly, no variables were of prominent importance, with the top nine variables ranging between VI = 0.05–0.08 (Fig. 3C). This suggests that none of the tested host and landscape variables were significant drivers of FIV_{Lru} SNP variation southeast of LA.

Discussion

Our wide sample distribution enabled us to quantify how host and landscape factors influencing FIV_{Lru} dynamics vary among local (i.e. either northwest or southeast of LA) and region-wide spatial scales. Patterns of FIV_{Lru} SNP variation region-wide, as well as more locally northwest of LA, were driven by geographic location, as evidenced by the significant effects of latitude and longitude in each of these analyses. At both scales, variation in land cover types among sample locations explained relatively little FIV_{Lru} SNP variation, except for the northwest of LA. Significant genetic co-structuring was observed between host and virus at the region-wide scale as well as in the northwest of LA but not in the southeast of LA. Analysis of FIV_{Lru} dispersal velocities revealed a weak positive effect of vegetation density on FIV_{Lru} spread at the region-wide scale, although there was no effect of landscape factors on dispersal velocities in areas northwest or southeast of LA individually. Under a landscape resistance analysis framework, we observed lower support for landscape drivers of dispersal velocities than under a least-cost paths framework.

Overall, rates of FIV_{Lru} dispersal were relatively slow (0.56–1.6 km/y) and consistent with previous estimates in these populations (see Fountain-Jones et al. 2017a) as well as in other urban wildlife pathogen systems. For example, rabies dispersal in urban dogs was 0.65 km/y compared with up to 22 km/y in non-urban areas (Bourhy et al. 2016; Dellicour et al. 2017). Urban development is an important factor limiting bobcat connectivity in coastal southern California (Riley et al. 2006; Ruell et al. 2012; Kozakiewicz et al. 2019), thereby reducing FIV_{Lru} gene flow to an extent that is observable at significantly smaller spatial scales than our study (Fountain-Jones et al. 2017a). Contrary to expectations, we found no direct relationship between urbanisation and either FIV_{Lru} dispersal or SNP variation. However, the observed positive effect of vegetation density on dispersal velocities, albeit weak, suggests that reductions in natural vegetation because of urban development may reduce rates of virus spread across the landscape. Although FIV_{Lru} diffusion and dispersal rates did not differ significantly between the northwest and southeast of LA, diffusion was significantly more variable in the southeast, potentially reflecting greater variation in urbanisation and habitat fragmentation across this area.

Our results indicate a strong association between FIV_{Lru} SNP variation and factors influencing bobcat population connectivity. Our ALE plots revealed specific genetic discontinuities among FIV_{Lru} isolates that coincide with major urban features. The most prominent of these discontinuities was evident in the region-wide analysis, located precisely between the latitudes and longitudes encompassing the LA basin. Previous work demonstrating associations between urbanisation and FIV_{Lru} genetic variation was conducted on some of the same individuals ($n = 11$, but using shorter length FIV_{Lru} sequences) but encompassed only a small section of our study area (southeast of and directly adjacent to LA) (Fountain-Jones et al. 2017a; Fountain-Jones et al. 2021). This scale and location dependence suggest that associations between fine-scale patterns of urbanisation and FIV_{Lru} SNP variation may differ among areas that contain similar amounts of urban development overall. For example, specific arrangements of suburban developments may influence the availability of movement corridors and locations where urban-associated features such as roads may be easily crossed. Such locally specific landscape associations have been demonstrated in the host, whereby environmental factors explaining bobcat gene flow differ among populations and also vary among different spatial scales (Kozakiewicz et al. 2019; Smith et al. 2020). This context-dependence makes generalisation challenging and highlights the difficulty of predicting landscape effects on pathogen transmission at fine spatial scales without locally specific data.

Northwest of LA, variation in the amount of forest land cover near sample locations influenced SNP variation among FIV_{Lru} isolates. This association was surprising, as forest comprises a very small proportion of overall land cover in this study area when compared to other vegetation types such as scrub/shrub.

This positive correlation implies an isolation by environment effect (Wang and Bradburd 2014), which can occur through factors such as selection against immigrants and habitat-biased dispersal (Wang and Bradburd 2014). However, selection derived from forest habitat is unlikely in coastal southern California given forest scarcity, necessitating frequent use of other habitat types by bobcats, usually scrub/shrub. Forest land cover northwest of LA is largely limited to the Santa Monica Mountains, which contain a bobcat population that is isolated from others by highways. Thus, the observed effect of forest land cover can likely be attributed to the effect of population isolation due to these highway barriers rather than to forest land cover itself (see Riley et al. 2006; Serieys et al. 2015; Kozakiewicz et al. 2019, 2020).

Co-structuring among FIV_{Lru} and host populations was evident through associations between FIV_{Lru} SNP variation and host genetic distances. Although the greatest host genetic distances were between the northwest and southeast of LA (PCo1), those within the northwest of LA (PCo2) were the most important predictors of FIV_{Lru} variation, even at the region-wide scale. Genetic distances among hosts between the northwest and southeast of LA were likely less important because these individuals were also more distant geographically, meaning that latitude and longitude explained a greater proportion of FIV_{Lru} SNP variation among these samples. In contrast, the northwest of LA contains genetically distinct yet geographically proximate bobcat populations (that are separated only by highways), resulting in the greater importance of host genetic distances in predicting FIV_{Lru} SNP variation and the reduced importance of geographic location. Ideally, each FIV_{Lru} population would be analysed separately, as was conducted for the host populations by Kozakiewicz et al. (2019), to account for the effect of population structure. However, sample size constraints given FIV_{Lru}'s relatively low prevalence did not permit such a design in this instance.

A relatively sparse sampling of highly divergent lineages southeast of LA (Supplementary Material 1, Fig. S2; also see Kozakiewicz et al. 2020) may have limited our ability to detect environmental associations with FIV_{Lru} SNP variation and lineage dispersal velocities. In addition, because FIV_{Lru} is a chronic infection, potential lags between the time of initial infection and the time of sampling may introduce additional noise to spatiotemporal dispersal estimates. Distantly related FIV_{Lru} lineages can circulate independently within the same host population and may co-infect the same individuals. Thus, differentiation between spatially proximate but highly distinct samples may be due to historical evolutionary processes rather than contemporary ecological factors. Furthermore, our analysis of FIV_{Lru} spread excluded phylogenetic branches that originated before 1980 to reduce the temporal mismatch between branches and contemporary land cover. These excluded branches often crossed areas of urban development, including the highly urbanised LA basin that separates our northwest and southeast study areas. Thus, our ability to detect the influence of these urban features on FIV_{Lru} dispersal velocities was likely reduced.

Compared to the least-cost path analysis, analysis under a landscape resistance framework suggested fewer landscape factors influencing FIV_{Lru} dispersal velocities. Landscape resistance analysis is commonly considered superior to least-cost path analysis because it can model all possible dispersal pathways available to an organism, as opposed to a single, optimal pathway that assumes organisms have complete knowledge of the landscape intervening sites. However, least-cost path analysis, in some cases, has been shown to perform well, potentially reflecting more linear structuring of landscape features (Schwartz et al. 2009; McClure, Hansen, and Inman 2016). The relatively constrained landscape in coastal southern California, with many impermeable barriers to bobcat movement such as coastlines, roads, and dense urban development, limits the number of potential pathways available to an individual bobcat infected with FIV_{Lru}. Thus, a model representing a single, specific pathway may be more representative of bobcat movement in this environment.

Our results are consistent with a growing body of research suggesting that broad constraints on host movements imposed by urban development constrain pathogen spread. Knowledge of these constraints may be leveraged to assist the management of infectious disease outbreaks, whereby known connectivity corridors may be targeted for surveillance or direct interventions. Yet, exploitation of such constraints must be balanced against the need for host population connectivity, the loss of which is a primary factor threatening wildlife globally (McCallum and Dobson 2002; Crooks et al. 2011). Importantly, our results suggest that urbanisation may play less of a role at fine spatial scales in constraining FIV_{Lru} transmission than it does to limit bobcat connectivity. Host genetic structuring may accurately predict pathogen spread, but genetic co-structuring between host and pathogen may only be evident when not confounded by deeper phylogenetic relatedness among pathogen lineages. Inconsistency with previous studies suggests that urban effects on virus transmission in bobcats may be dependent on spatial scale or other locally specific factors, highlighting the difficulty of generalising these findings. However, due to the broad distribution of bobcats and FIV_{Lru} in North America across a variety of environments (Reding et al. 2012; Lagana et al. 2013; Carver et al. 2016), this system represents an excellent opportunity to investigate factors driving pathogen transmission in a variety of landscape contexts. Ultimately, this work demonstrates the potential of emerging ecological phylogenetic and machine learning approaches in elucidating the factors shaping pathogen transmission in urban landscapes. With the continued expansion of urban landscapes globally, understanding how urbanisation impacts patterns of pathogen transmission will be increasingly valuable.

Data availability

Data, including sample metadata and sequence accession numbers, are available in Supplementary Material 2.

Supplementary data

Supplementary data are available at *Virus Evolution* online.

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