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Hunting alters viral transmission and evolution in a large carnivore

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Hunting can fundamentally alter wildlife population dynamics but the consequences of hunting on pathogen transmission and evolution remain poorly understood. Here, we present a study that leverages a unique landscape-scale quasi-experiment coupled with pathogen-transmission tracing, network simulation and phylodynamics to provide insights into how hunting shapes feline immunodeficiency virus (FIV) dynamics in puma (*Puma concolor*). We show that removing hunting pressure enhances the role of males in transmission, increases the viral population growth rate and increases the role of evolutionary forces on the pathogen compared to when hunting was reinstated. Changes in transmission observed with the removal of hunting could be linked to short-term social changes while the male puma population increased. These findings are supported through comparison with a region with stable hunting management over the same time period. This study shows that routine wildlife management can have impacts on pathogen transmission and evolution not previously considered.

Human actions commonly alter wildlife populations. A classic example is hunting, which often has density and demographic effects on a population[1–4]. Recreational quota-based hunting of carnivore populations is common across the globe[5,6], however, the consequences of these actions on pathogen transmission and evolution are largely unknown and the few available studies report contradictory findings. Theory predicts that for pathogens with density-dependent transmission, hunting-induced reductions in density should decrease transmission rates, yet make little difference to transmission dynamics for frequency-dependent pathogens. Empirical data and models suggest that reducing host density can either decrease[7,8], have no effect[9] or even increase pathogen transmission and prevalence[10]. The complex interplay between host density, demography and behaviour makes predicting the impacts of hunting on pathogen dynamics difficult.

Human harvest of wild populations is often non-random (for example, a preference for large males[1] or a particular behaviour[11]) and if different sexes, ages or behavioural types contribute disproportionately to disease transmission, this could have implications for disease dynamics[12]. Empirical work shows that population reduction can increase pathogen prevalence via social perturbation[13–17]. For example, culling-induced changes or 'perturbations' to badger (*Meles meles*) territorial behaviour was considered a driver of increased bovine tuberculosis transmission among badgers[13,17,18]. Culling male badgers may be particularly important, as male–male contact networks are structured over larger spatial scales that potentially facilitate between-group spread[17,18] However, there is also evidence that population reduction has little impact, such as is seen for canine rabies[19] and Tasmanian devil facial tumour disease[20] dynamics. Recent advances in high-resolution pathogen sequencing and analytic approaches can now elucidate patterns of pathogen transmission and evolution[21–23] that were previously out of reach. Here, we address the effects of hunting on pathogen dynamics by capitalizing on pathogen sequences collected from a detailed study on the demographic effects of hunting[24] as well as from sequences obtained over the same time period in a region where little hunting occurred. Our approach enables us to provide insights into the cascading consequences of hunting and the cessation of hunting on host–pathogen dynamics.

RNA viruses are ideal agents for examining the effect of hunting and the cessation of hunting on pathogen transmission and evolution. Genomic variation rapidly accrues in RNA viruses, enabling estimation of fine-scale epidemiological processes (such as transmission between hosts) and the basic reproduction number (R_0)[22,25] (see Table 1 for definitions of key terminology). Altered transmission dynamics and the arrival of new lineages can imprint distinctive evolutionary signatures on RNA viruses as they adapt quickly to changes in host populations they encounter[26,27]. For example, if a change of management that increases contact rates leads to a higher frequency of transmission events, the transmission bottleneck may lead to high purifying selection since within-host mutations are lost with transmission (for example, ref. [28]). Conversely, if new mutations entering the host population, for example, via increased host immigration, allow the pathogen to escape immune detection,

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Table 1 | Description of key social network, transmission tracing and phylodynamic terminology used in the manuscript

Term	Definition
R_0	The basic reproduction number '*R* naught' is the expected number of cases generated by one case in a population of susceptible individuals.
Transmission bottleneck	Transmission of viruses between hosts usually involves a relatively small number of virus particles being exchanged between hosts (for example, ref. [52]). This has the effect of reducing viral genetic diversity population size and creating a 'bottleneck'.
Purifying selection	'Negative selection' is the removal of non-synonymous mutations (mutations that lead to a change in protein coding).
Diversifying selection	'Positive selection' is the favouring of non-synonymous mutations that yield an adaptive advantage. These mutations can rapidly increase in frequency across a population.
Transmission network	A network where nodes represent individual puma and edges reflect transmission events based on transmission tree estimates. Edge weights are the probability of the transmission event occurring. Transmission trees generated by the R package TransPhylo[22] estimate who infected whom, including potentially unsampled individuals using a stochastic branching epidemiological model and a time-scaled phylogeny.
Weighted degree	The summed probability of a individual puma (a node in the network) being involved in transmission events divided by the number of transmission events (edges in the network).
Weighted degree homophily	The weighted degree of transmission events between members of the same sex.
Skygrowth demographic analyses	Non-parametric population-genetic model estimating the growth effective population size through time (a surrogate for genetic diversity) using Bayesian inference. This method has been shown to accurately reconstruct pathogen outbreak dynamics in a variety of systems[45,66].

we may expect an increase in diversifying selection. Altered transmission dynamics and new lineages will also shape the phylogenetic diversity of the pathogen[29]. For example, if novel pathogen lineages are frequently arriving into a host population with limited transmission, we would expect to see a pattern of phylogenetic dispersion (higher phylogenetic diversity than expected by chance[30]). In contrast, phylogenetic clustering (lower phylogenetic diversity than expected by chance[30]) may be a marker of increased transmission events within a population.

Here, we leverage cross-sectional viral data collected from closely monitored puma (*Puma concolor*) in two areas in Colorado during the same time period: a 'treatment region' in which hunting pressure changed over time and a 'stable management region' acting as a control (hereafter, 'stable region'). We sequenced viral genes sampled from captured puma for an endemic RNA retrovirus, puma feline immunodeficiency virus (FIV_{pco}), which is a host-specific pathogen considered relatively benign and not associated with overt disease outcomes[31]. FIV_{pco} is a lifelong infection that is not eliminated by sterilizing immunity and is endemic in most puma populations[32]. Previously infected individuals can also become infected with new FIV_{pco} strains[33]. As apex predators, puma occur in low density and contact between adults (and potential transmission events) occurs mainly via mating or during territorial fights among males (although contact around food resources may be more common than previously thought[34]). After becoming independent from their mothers at between 10 and 20 months of age, males nearly always leave their natal range whereas 50–80% of female offspring set up adjoining home ranges[35]. Evidence suggests that FIV_{pco} is often transmitted via aggressive interactions, although vertical transmission is also possible[31,36].

We analysed FIV_{pco} data from treatment and stable management regions using a transmission network approach[22,29] that incorporates a stochastic epidemiological model with pathogen genomic data to trace transmission between individual puma. When we combined the viral data with field observations and host genomic data, this approach enabled us to quantify differences in transmission networks associated with hunting and to characterize putative transmission events. The types of aggressive interactions that are transmission relevant are largely unknown (but see refs. [37,38]); however, on the basis of our understanding of puma behaviour[39] we suggest the following: (1) a dominance of within-sex transmission should indicate that competition for mates or resources is important; (2) a preponderance of transmission events between related males and females may be indicative of familial transmission and/or vertical transmission; and (3) transmission primarily occurring between unrelated males and females may indicate interactions associated with mating may be important.

Results and discussion

We constructed FIV_{pco} transmission networks in both study regions. The treatment region consisted of puma in an ~12,000 km^2 area in western Colorado in which hunting before our study was common practice (see ref. [24]). Hunting was excluded for a 5-year period (November 2004–November 2009, 'no-hunting period') and reinstated for a further 5 years afterwards (November 2009–March 2014, 'hunting period'). The harvest rate averaged 15% of the independent puma that used the study area across this 5-year hunting period, with males favoured by hunters (32 of the 46 pumas killed were adult males[24]). Hunting was excluded in this region to facilitate a study on the population-level effects of regulated hunting on puma in Colorado[24]. During the no-hunting period in the treatment region, the population of independent pumas (adults and subadults) increased from an estimated 23 (2005) to 57 (2009) individuals, with much of this growth occurring between 2007 and 2010[24] (after a 2-year lag during 2004–2006, hereafter 'lag 1'). Adult and subadult male survival was significantly higher in the no-hunting period[24] and we suggest that this may increase transmission events associated with competition for mates. When hunting resumed in November 2009, the overall population declined after a lag of 2 years with male abundance estimates similar to the start of the no-hunting period (2009–2011, hereafter 'lag 2'; Supplementary Table 1). However, the decline in abundance of males was severe and rapid with males >6 years old apparently eliminated from the population after two hunting seasons (mortality rates from other sources such as vehicle strike in both periods were similar[24]). See Supplementary Tables 1 and 2 for a summary of abundance and FIV_{pco} data. In contrast, over the same 10-year period, the stable region in the Front Range of Colorado experienced continued minimal hunting pressures (three individuals killed from 2007 to 2013[40]) and no change in management practice. Previous genetic analysis revealed that the puma in

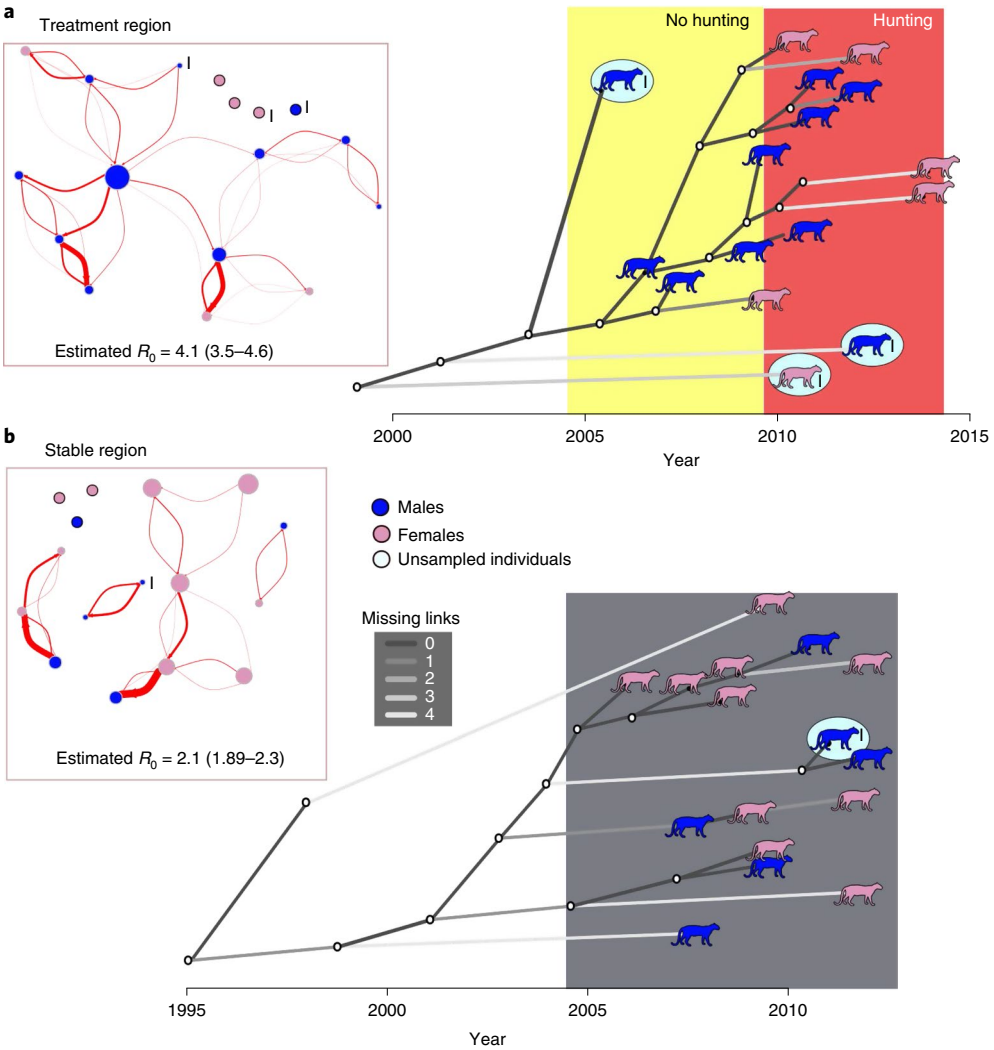

Fig. 1 | The transmission of FIV_{pco} was dominated by males in the treatment region, whereas females were more central in the stable management region. a,**b**, Transmission networks (left) and transmission trees (right) are shown for the treatment (**a**) and stable management regions (**b**). Males, blue nodes/puma silhouettes; females, pink nodes/puma silhouettes. Nodes connected to each other via edges indicate the probability of transmission in either direction. Node size in the networks (left) is scaled on the basis of the number of edges estimated for each individual. Edge width is scaled according to the probability of the transmission events, where wider edges indicate a more likely transmission event (Extended Data Fig. 1). R_0 estimates (with 95% highest posterior density) are based on the stochastic branching epidemiological model underlying each transmission network (Methods; ref. [22]). Transmission trees (right) show these putative transmission events through time with branch colour indicating how many missing edges are likely between individuals. Yellow background, hunting pressure relieved; red background, hunting pressure resumed; white nodes, unsampled individuals estimate by the model; I, individuals that were probably immigrants in this region on the basis of ref. [41]. See Extended Data Fig. 2 for the FIV_{pco} generation time distributions for each region and Extended Data Fig. 3 for the estimate of missing cases across year.

these regions were genetically distinct with few clear migrants[41]. Nearly all the individuals sampled in both regions were adults and both sexes were evenly represented. Individual survival probabilities in the stable region were unaltered across years[40]. By comparing the treatment and stable regions, we were able to test how demographic changes, including heterogeneity in survival between the sexes, caused by hunting cessation and reinstatement, perturb viral transmission networks, epidemiological parameters (for example, R_0) and pathogen diversity and evolution. In doing so, we begin to untangle the complex interplay between wildlife management and pathogen transmission, which is crucial for pathogen-orientated conservation and disease management strategies.

Cessation of hunting shifts transmission networks and increases R_0. We found that reducing hunting mortality had major effects on FIV_{pco} transmission dynamics. Even though the regions were of comparable geographic size and contained similar puma abundance (Supplementary Table 2), our estimates of R_0 for the same virus over the 10-year period were twofold higher in the treatment region compared to the stable region (with non-overlapping 95% high probability density intervals indicating that the difference is significant, Fig. 1; see Supplementary Table 3 for sensitivity analysis results). Other model parameters, such as generation time (the time between initial FIV_{pco} infection and onward transmission; Extended Data Fig. 2) and the proportion of missing cases (Extended Data Fig. 3), yielded similar estimates in both regions. The burst of transmission in the treatment population after the cessation of hunting (Fig. 1a, right) was probably a result of transmission between males as they were dominant in the network. In the treatment population, males had an overall mean weighted degree (Table 1) double that of females

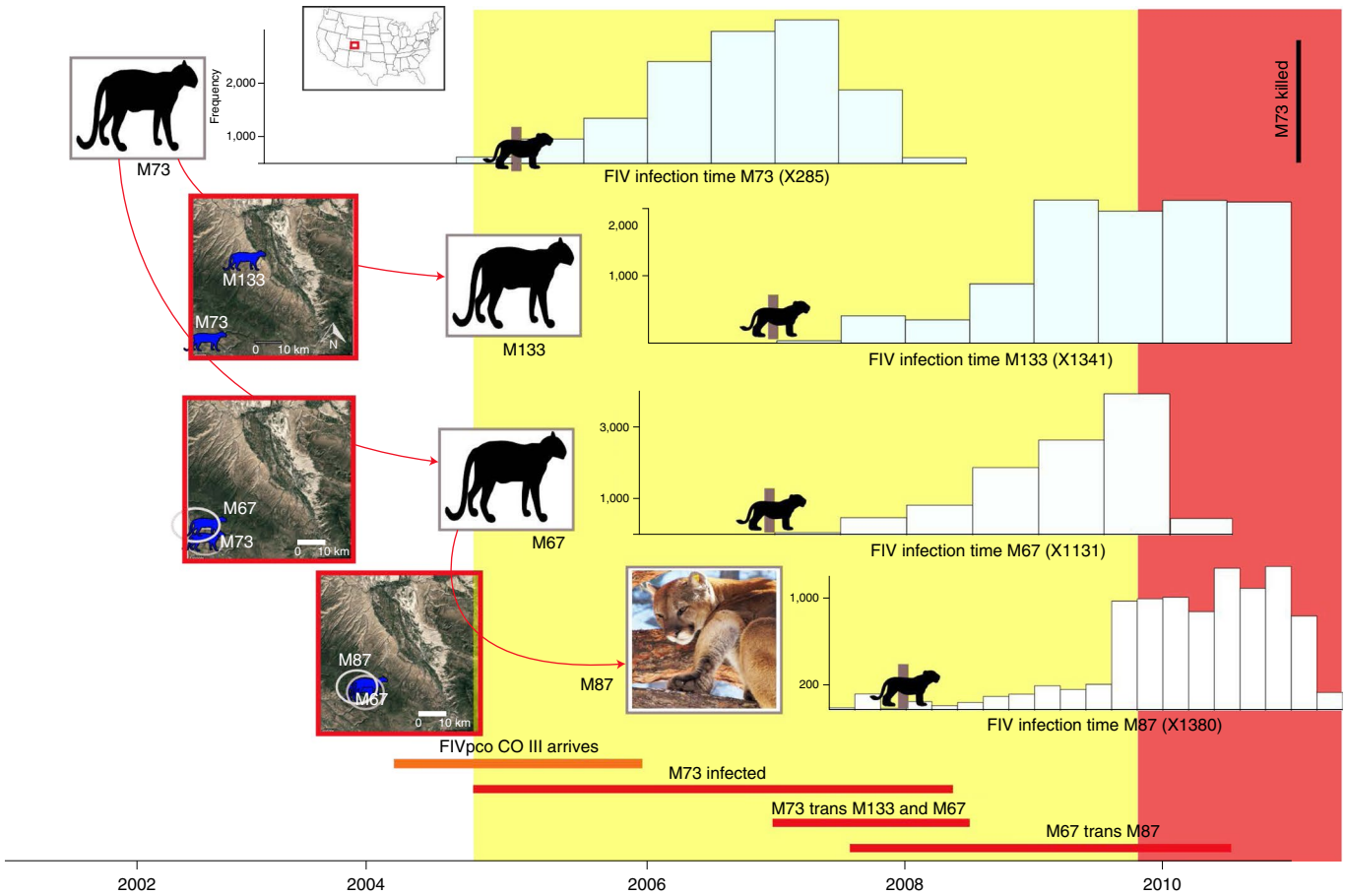

Fig. 2 | Predicted FIV_{pco} transmission events and their estimated timing among puma involved in a transmission chain. Infection time distributions among an illustrative group of pumas from our transmission network model (Fig. 1). Red arrows show the likely direction of transmission and the maps show the spatial context (see Extended Data Figs. 5 and 6 for information on other transmission events in the treatment and stable region, Map data: Google ©2020). Grey ovals encompassing puma silhouettes in the map insets represent known territorial overlap between individuals (based on unpublished radiotelemetry location estimates from K.L.) and are not representative of territory size. Light yellow, hunting pressure relieved. Birth year is indicated by the cub silhouette and death year of M73 is indicated by the black horizontal line. The orange horizontal line indicates when the FIV_{pco} CO III lineage was introduced into this population on the basis of node estimates from ref. [38]. Red horizontal lines indicate transmission time distributions (overlap between infection time distributions) and 'trans' means 'probably transmitted to'. Photo of M87 was taken by K.L. See Extended Data Fig. 7 for the locations of all individuals sampled.

(0.23 compared to 0.08). Only one putative transmission event occurred between sexes and we detected no female–female transmission events in the treatment region. When we assessed weighted degree homophily of male–male transmission events, simulations revealed that the dominance of male–male transmission events in the network was not random (1,000 simulated annealing network iterations, $P<0.001$; Extended Data Fig. 4a and Supplementary Table 3). Putative transmission events largely occurred when hunting mortality was eliminated (Fig. 1a), during which time the survival of adults and subadult males was high, average age increased and the abundance of independent pumas increased[24]. During the hunting period, male survival rates were lower than for either sex in the stable region[24]. Female survival was also reduced in the hunting period but the decline was not as dramatic as it was for males[24]. Females were, however, much less connected in the transmission network in the treatment region compared to the stable region, where they were more connected (Fig. 1b). In contrast to the treatment region, the stable region showed evidence of transmission from females to both females and males. Average weighted degree was higher overall for males than for females in the stable region (0.46 versus 0.29). Weighted female–female degree homophily was similar in both regions (0 in the treatment region versus 0.05 in the stable region, $P=0.692$; Extended Data Fig. 4b and Supplementary Table 3). Female-to-female transmission events in the stable region occurred between highly related females, supporting previous findings of the importance of host relatedness in FIV_{pco} spread for puma in this region[38]. Taken together, our results indicate that lower hunting mortality was associated with an increase in the number of transmission events which were dominated by males.

After hunting was prohibited, the greater survival and increasing abundance of males probably resulted in greater competition between males for mates[24]. As the dominant transmission mode for FIV_{pco} is considered to be via aggressive contacts[42], increased male competition for mates appears a probable explanation for the differences in transmission dynamics. Further interrogation of our transmission network supports this theory, as in all but two instances, male-to-male transmission occurred between individuals with overlapping territories in the treatment region (Fig. 2 and Extended Data Figs. 5 and 6; based on our radiotelemetry location data (K.L., unpublished data)). One transmission pair was unusual in having less spatial proximity, yet one puma of this pair was a likely immigrant to the region (M133) and could have passed through M73's territory at some point (Fig. 2). With the exception of M73 (~6 years old at time of infection), all individuals involved in these transmission events were between 1 and 3 years old, which is a period when males are establishing territories and are starting to compete for access to

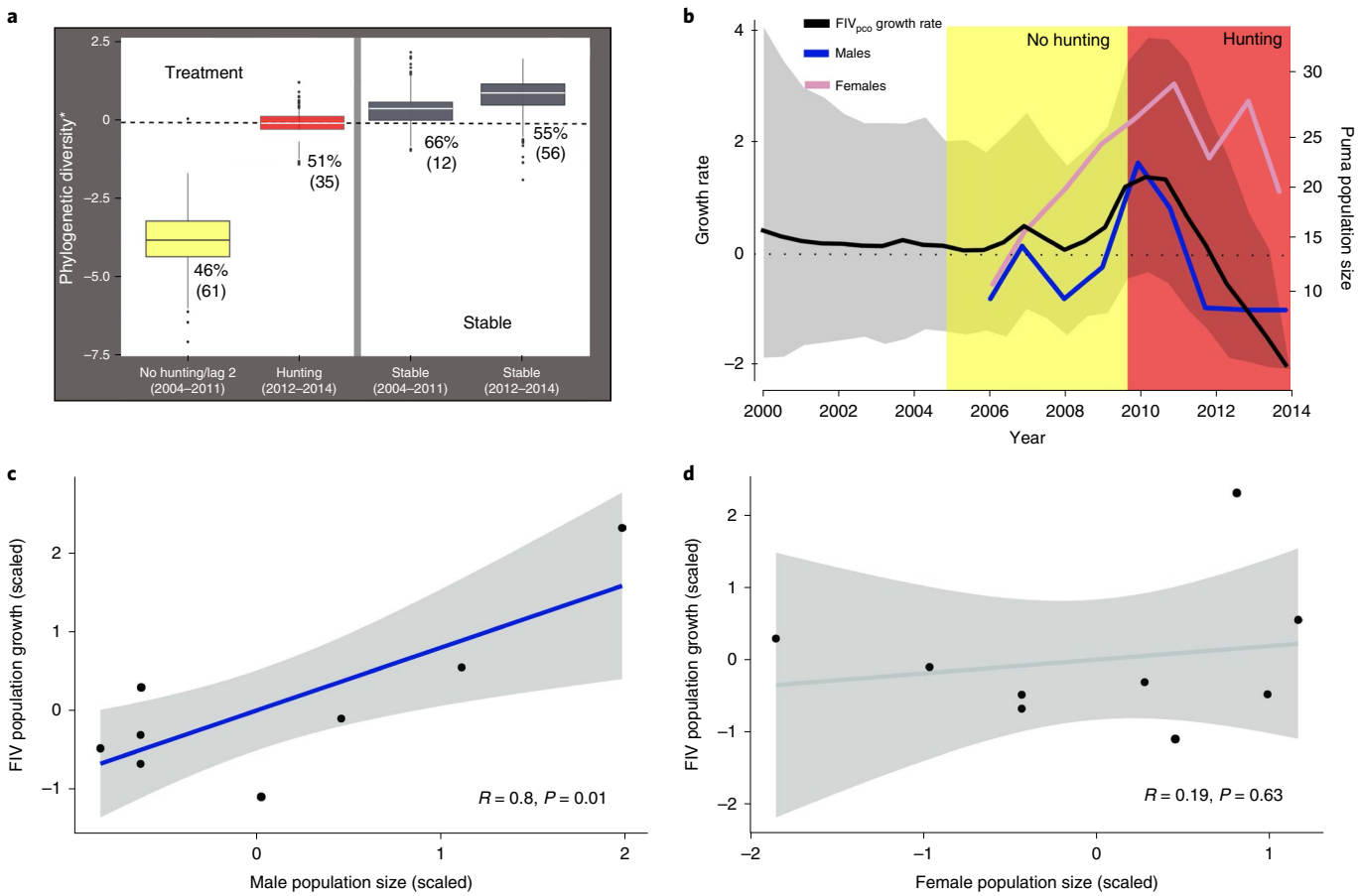

Fig. 3 | The elimination of puma hunting in the treatment region led to an increase in FIV$_{pco}$ diversity that correlated positively with male population size. **a**, Standardized phylogenetic diversity of FIV$_{pco}$ from puma in the treatment and stable regions, showing reintroduction of hunting within the treatment area was associated with FIV$_{pco}$ diversity increasing to be comparable to the stable region. **b**, Temporal trends in estimated growth rate of FIV$_{pco}$ (black line) in the treatment region during no-hunting (yellow shading) and hunting (red shading) periods and associations with estimates of population size of male (blue line) and female (pink line) puma. **c**,**d**, Scatter plots showing that FIV$_{pco}$ population growth rate was positively correlated with male population size (**c**) but unrelated to female population size in the treatment region (**d**). Note that in **a**, * is the standardized effect size for phylogenetic diversity (SES.PD) calculated from 1,000 posterior trees; estimated FIV$_{pco}$ prevalence (number of qPCR positives/total number sampled) is provided next to each box; the number of individuals tested is shown in parentheses (see Extended Data Fig. 9 for estimates of prevalence across years); sequences from puma sampled in the lag 2 period were included in the no-hunting period; and there was one sequence sampled in the lag 1 period and this was retained in the hunting period as it made no difference to the diversity estimate. Note that in **b**, viral population growth rate was estimated using Bayesian phylodynamic reconstruction[45]; the dashed horizontal line reflects the 0-growth line; and see Extended Data Fig. 8 for the corresponding skyline plot and Extended Data Fig. 8 for complementary plots for the FIV$_{pco}$ clade dominant in the stable region (effective population size through time estimated via the *phylodyn* model[67]).

females[39,43]. Our results suggest that it is unlikely that these males transmitted to each other before dispersal or via maternal or paternal contacts—since these individuals were not related on the basis of genomic data[41]. While our estimates suggest that we were able to sample ~40% of the FIV$_{pco}$ infections in both regions (Supplementary Table 1 and Extended Data Fig. 3)—arguably good coverage for secretive, free-ranging wildlife—our models account for this type of missing data[22]. For example, nearly all putative transmission events we identified from our transmission networks were between individuals on the landscape at the same time and in most cases were captured in close spatial proximity to each other. The biological plausibility of these transmission events demonstrates the power of adapting transmission network models to trace transmission and gain epidemiological insights in systems that are difficult to observe.

Hunting alters diversity and selective pressure on the virus. Altered transmission dynamics at a population level were associated with changes in viral evolution and diversity in the treatment region. The increased number of transmission events in the no-hunting period compared to the hunting period was supported by the strong phylogenetic clustering (isolates with less phylogenetic diversity than expected by chance) detected relative to the hunting period (Fig. 3a). While not directly quantified here, differences in intrahost evolutionary rates are unlikely to explain regional differences in phylogenetic diversity as FIV intra-individual evolution rates have been found to be stable across hosts and are roughly equivalent to FIV interindividual rates[44]. This supports the idea that the demographic changes associated with hunting, rather than intra-individual variation, are likely to shape the viral phylogenetic patterns observed. The link between reduced hunting pressure and increased transmission events was further supported as we did not find similar phylogenetic clustering in the stable region or hunting period (Fig. 3a). Moreover, we found little evidence for new lineages arriving during the no-hunting period in the treatment region (Fig. 1a). We further interrogated viral diversity patterns across time using skygrowth demographic analyses[45]. Viral genetic diversity rapidly accrued at the end of the no-hunting period (~2009/2010) before markedly declining after ~2011 when hunting was reinstated

(Fig. 3b), closely mirroring male population size estimates ($R^2 = 0.8$, $P = 0.010$, Fig. 3c). In contrast, female population size was not significantly correlated to viral population growth rate ($R^2 = 0.190$, $P = 0.630$, Fig. 3d). Collectively, the relationships between host abundance estimates and viral population growth rates support a greater role of male interactions in transmission dynamics across hunting intensities, relative to females. While we lack behavioural observations of puma across time, it is possible that the increase in male density with the cessation of hunting allowed for increased competition for mates and thus aggressive interactions[43]. No such increase in FIV_{pco} diversity and growth rate was detected in the stable population (Extended Data Fig. 8).

Within the treatment region, the increase in viral diversity was underpinned by greater effects of both purifying and diversifying selection acting on viruses that infected individuals during the no-hunting period compared to the hunting period ($P = 0.01$, likelihood ratio = 6.31). Purifying selection, potentially as a signature of rapid transmission events (for example, ref. [22]), was dominant in both periods (97.25% sites $\omega < 1$), as is often the case in error-prone RNA viruses, but stronger in the non-hunting period than the hunting period ($\omega_{2nh} = 0$, $\omega_{2h} = 0.1$; nh = non-hunting, h = hunting). In contrast, there was no shift in evolutionary pressure in the same periods in the stable population ($P = 0.5$, likelihood ratio = 0.43). While impacting a smaller proportion of the loci overall (2.79% loci $\omega > 1$), there was strong diversifying selection in the no-hunting period as well ($\omega_{3nh} = 21.46$, $\omega_{3h} = 2.8$). Using the mixed effects model of evolution (MEME) routine that tests for selection at individual sites on a proportion of branches[46], we identified five FIV_{pco} loci under diversifying selection in both regions (cutoff: $P \leq 0.1$). Two of the sites had non-synonymous substitutions just in isolates in males and, on the basis of our transmission models, the males were probably infected by FIV_{pco} in the no-hunting period. There was no signature of diversifying or purifying selection in the envelope gene (*env*), which was surprising given that *env* is generally under greater evolutionary pressure as it is responsible for the virus binding to the host cells[47]. All loci under diversifying selection were detected in the FIV *pol* integrase region. Putting these lines of evidence together, we not only detected population-level impacts of demographic changes due to cessation of hunting on viral mutation but also at the individual scale with stronger evolutionary pressure on viruses infecting males. Increased evolutionary pressure on the virus may increase the probability of a new FIV_{pco} phenotype occurring in this population. Systematic shifts in evolutionary pressure are known to occur when viruses switch hosts[48,49]; however, here we show that selective constraints on a virus can be altered in response to host demographic changes caused by wildlife hunting.

Perturbation, management and disease. Our work provides a valuable case study on how changing hunting pressure can have unexpected consequences for pathogen transmission and evolution across scales. Our analytical approach was particularly valuable in helping to deconstruct how shifts in population structure imprint on pathogen dynamics and evolution. For example, previous work using landscape genetic models only detected weak or inconsistent sex effects shaping FIV spread[36,38,50]. Our transmission network and phylodynamic approach, in contrast, was able to clearly distinguish the role of males in putative transmission chains and in accruing genetic diversity, even though the data requirements are similar (for example, a time-scaled phylogeny). Scale dependence may be one reason for the difference, as landscape genetic approaches obscure individual transmission events while quantifying the population-level signature of host and landscape on pathogen spread[51]. The putative transmission events we detected, supported by observational data, provided important mechanistic details at an individual scale that enabled us to tease out the links between management, sex and transmission that are difficult to detect otherwise. The shift in connectivity within the observed transmission network between sexes provided context to the differences in pathogen evolution we detected between the no-hunting and hunting periods. Our study provides new dimensions to the importance of understanding sex-specific variation when managing infectious disease in wildlife[17,52]. We stress that our findings are specific to FIV in puma but also note that there is growing evidence that chronic lifelong retroviral infections, such as FIV, may be a useful apathogenic proxy for other directly transmitted and more virulent pathogens[37,53].

Our results provide a case study of the complex interplay between wildlife management and demography in shaping pathogen dynamics. In our case, the cessation of hunting in a region facilitated demographic change via increased male survivorship and abundance[24], with potential increases in male-to-male contact behaviour. Even though the 'perturbation' was the cessation of hunting, the underlying mechanism could be similar (for example, lead to demographic and behavioural shifts that increase transmission). An expansion in the way we think about perturbations to include a cessation of a practice leading to demographic or behavioural change may be warranted. Any period of perturbation (intended or otherwise) to the demographic structure of a wildlife population for which disease poses a major threat (for example, the Florida panther[33] and Tasmanian devils[12]) may warrant additional pathogen surveillance and potential disease mitigation plans.

Our results also reveal potential limitations of population estimates of prevalence to understand the impact of wildlife management actions on pathogen transmission. In our case, population estimates of FIV_{pco} prevalence across time alone could not detect shifts in transmission associated with hunting and were not sensitive to changes in population size (Extended Data Figs. 9 and 10). The lack of signal from prevalence data may be a contributing factor behind the variability of the effects of hunting on disease dynamics in empirical systems. Prevalence data may be better able to detect shifts in population demography where the pathogen causes acute infections with shorter periods of immunity.

Conclusions

The collection of pathogen molecular data from well-sampled wildlife populations across time is a logistical challenge yet, with ever cheaper and more mobile sequencing platforms, the potential to use approaches similar to ours is increasing, even for slowly evolving pathogens such as bacteria[25]. Our approach can not only provide new insights into the broader consequences of wildlife management on disease dynamics but can also help understand evolutionary relationships between hosts and pathogens in free-ranging species more broadly.

Methods

Study area and puma capture. Our study was conducted in two regions in the Rocky Mountains in Colorado separated by ~500 km but at similar elevations and with similar estimates of puma abundance[24,54], vegetative and landscape attributes, yet with differing degrees of urbanization (Extended Data Fig. 7 and ref. [55]). In the treatment region in the Uncompahgre Plateau on the Western Slope of Colorado, blood samples were taken from 114 individuals (Supplementary Table 1) and monitored intensively (with very high frequency radio and GPS collars) until their death or the end of the study in 2014. In the stable management region in the Front Range of Colorado, blood samples were taken from 56 individuals from 2005–2014. Captured pumas were anaesthetized with established sedative and tranquilizer protocols[24] and released after blood, serum and oral swabs were collected. Animal sex, age and capture location were recorded. See ref. [38] for sample storage, FIV_{pco} DNA extraction and sequencing details. In brief, for samples that were by quantitative PCR (qPCR) positive for FIV_{pco}, the complete *ORFA* and *pol* gene regions were isolated using a nested PCR protocol[38]. Recombination was removed and the genes were concatenated together. See Supplementary Tables 1 and 2 for a summary of the sequence data and a comparison of study area size, estimates of host abundances, host mortality and host genetic diversity between regions. All puma were handled in accordance with approved Colorado Parks and

Wildlife (CPW) Animal Care and Use Committee (ACUC) capture and handling protocols (ACUC file no. 08-2004, ACUC protocol no. 03-2007 ACUC 16-2008).

Transmission and phylogenetic trees. We constructed transmission trees between pumas in each region using the R package *TransPhylo*[22]. *TransPhylo* uses a time-stamped phylogeny to estimate a transmission tree to gain inference into 'who infected whom' and when. Briefly, this approach computes the probability of an observed transmission tree given a phylogeny using a stochastic branching process epidemiological model; the space of possible transmission trees is sampled using reversible jump Markov chain Monte Carlo (MCMC)[22]. This approach is particularly useful for pathogens where the outbreak is ongoing and not all cases are sampled[22], as is the case here. We leveraged our FIV_{Pco} Bayesian phylogenetic reconstructions from previous work and focused on the two clades of FIV_{Pco} that predominantly occurred in each region (ref. [38]). Whilst the TransPhylo approach makes few assumptions, a generation time distribution (the time from primary infection to onward transmission) is required to calibrate the epidemiological model[22]. We assumed that generation time could be drawn from a gamma distribution ($k=2$, $\theta=1.5$) estimating onward transmission on average 3 years postinfection (95% interval: 0.3–8 years, based on average puma age estimates[43]). On the basis of previous work[24,55] (see Supplementary Table 1 for treatment region estimates), we were confident that the proportion of cases (π) sampled was high, therefore we set the starting estimate of π to be 0.6 (60% of cases tested in each region) and allowed it to be estimated by the model. We ran multiple MCMC analyses of 400,000 iterations and assessed convergence by checking that the parameter effective sample size (ESS) was >200. We computed the posterior distributions of R_0, π and the realized generation time from the MCMC output. We also estimated likely infection time distributions for each individual and compared these estimates to approximate puma birth dates to ensure that these infection time distributions were biologically plausible. We then computed a consensus transmission tree for each region to visualize the transmission probabilities between individuals through time. Lastly, we reformatted the tree into a network object (nodes as individual puma and edges representing transmission probabilities) and plotted it using the *igraph* package[56] and overlaid puma sex as a trait. Overall weighted degree and weighted degree for each sex, including edges representing homophily (for example, male–male) and heterophily (for example, male–female), were also calculated using igraph. See ref. [57] for more details on the *TransPhylo* pipeline.

To test the sensitivity of our results, we reconstructed transmission trees using the TransPhylo approach above but randomly dropping a tip from each $FIV_{pco,}$ phylogeny in each region. As running this transmission tree approach is computationally demanding, we performed ten iterations and summarized our estimates of R_0 and weighted degree homophily.

Simulation modelling. To test for non-random patterns of weighted degree between each sex, we applied a simulated network annealing approach from the Ergm R package[58]. To generate each simulated network, we fitted a variety of probability distributions to edge weight and degree of the transmission networks in both treatment and stable regions, then used Akaike information criterion to select the best-fitting target distribution. We were unable to subdivide the treatment region into hunting and no-hunting periods as there were no transmission events detected to help parameterize the models after 2012. Edge density, network size and the number of isolated nodes were fixed on the basis of each observed network. We added sex to each simulated node attribute drawing from a Bernoulli distribution ($P=0.5$). Using these network characteristics, we generated 1,000 'null' networks and compared the homophily weighted degree distribution of each sex (that is, the average weighted degree for each individual based on putative male–male or female–female transmission events) of the null networks to the observed and calculated a bootstrap *P* value.

Selection analyses. To test if the demographic changes driven by hunting resulted in a reduction in the intensity of natural selection on FIV_{pco}, we examined selective pressure in both time periods in each region using the RELAX hypothesis testing framework[59]. The method builds on random effects branch-site models (BS-REL)[60] that estimate the ω ratio (the ratio of non-synonymous to synonymous mutations or d*N*/d*S*) along each branch from a discrete distribution of three ω ratio classes, allowing selection pressure to vary across the phylogeny[59]. A ω ratio of one corresponds to neutral selection with values >1 being evidence for diversifying (positive) selection along a branch and <1 evidence for purifying (negative) selection along a branch. Briefly, RELAX tests for relaxation of selection pressure by dividing branches into three subsets: test branches (T), reference branches (R) and unclassified branches (U)[59] with ω_T (or ω_R) being the estimated d*N*/d*S* ratio on test (or reference) branches. The discrete distribution of ω is calculated using BS-REL for each branch class and then branches belonging to each subset are compared. The reference estimates of ω are raised to the power of k (an intensity parameter) so that $\omega_T = \omega_R^k$ to simplify model comparison. The null RELAX model is when the ω distribution and thus selective pressure is the same in R and T (when $k=1$). The null model is compared to an alternate model (using a likelihood ratio test) that allows k to vary so that when $k>1$ selection pressure on the test branches was intensified or $k<1$ indicating that selection pressure has been relaxed[59]. In the relaxed scenario, $k<1$ branches in R are under stronger purifying and diversifying selection compared to T branches (for example, ω shifts from 0.1 to 0.001 or from 10 to 2). See ref. [59] for model details. T and R were selected from leaf branches (all other branches were unassigned, U); individuals sampled from 2005 to 2011 (to the end of the lag period) were assigned to the R set and those sampled from 2012 to 2014 were assigned to the T set. All branches not directly connecting to the tips were classified as 'U' as the majority had low phylogenetic support (posterior probability <0.6). To further interrogate the sequence data to identify individual sites under selection, we performed the MEME pipeline[46]. For the putative sites under selection, we scanned the alignment to help determine which lineages/hosts accrued infections with these non-synonymous substitutions. We performed both MEME and RELAX models using the Datamonkey web application[61].

Population growth rate. We applied the non-parametric skygrowth method[45] to examine if the FIV_{Pco} population growth rate fluctuated across time and if this was related to changes in male or female population size in the treatment region. We did not relate puma population sizes to FIV_{Pco} growth rates for the stable region, as similar host population size estimates through time were not available. We fitted these models using MCMC (100,000 iterations) assuming that FIV_{Pco} population size fluctuated every 6 months over a 14-yr period (the estimated time to most recent common ancestor of this clade; Extended Data Fig. 8). Otherwise, the default settings were used. We then performed a Pearson correlation test to assess if the trend in FIV_{Pco} population growth was related to male and female population size estimates[24]. Measuring the correlation between population size estimates and patterns of population growth using generalized linear models[45,62] was not feasible due to the relatively small size of this dataset.

Phylogenetic diversity. To quantify phylogenetic diversity in each time period in each region, we calculated the standardized effect size (SES) for Faith's phylogenetic richness that accounts for differing sample sizes (SES for Faith's PD[63]). Faith's PD (hereafter, PD) is the sum of the branch lengths of the phylogenetic tree linking all isolates for each subset (in this case, the two time periods). As the number of isolates in each contrast differed (stable region during 2005–2011, 11 isolates; stable region 2012–13, five isolates; treatment region 2005–2011, ten isolates; treatment region 2012–14, five isolates), we calculated the SES by comparing the PD we observed to a null model that accounts for number of tips (that is, how much phylogenetic diversity would we see for a given number of isolates by chance). We denote the standardized PD as SES.PD from here on; this was calculated across a subset of posterior phylogenetic trees from our previous Bayesian phylogenetic analyses[38]. To capture phylogenetic uncertainty in these estimates, we used the computational efficiency of the *PhyloMeasures* R package algorithm[64] to calculate SES.PD and apply this across a 1,000 tree subsample of posterior trees[38]. Specifically, for each calculation of SES.PD we compared our observed PD to a uniform null model (that is, isolate samples are taken with equal (uniform) probability).

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

DNA sequences are GenBank accessions MN563193–MN563239. All other data and code to perform the analysis are available on Github at https://github.com/nfj1380/Transmission-dynamics_huntingPumaFIV[65]

Code availability

The code and data to perform these operations as well as the transmission tree analysis above can be found here: https://github.com/nfj1380/Transmission-dynamics_huntingPumaFIV[65]

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Author contributions

N.M.F.J. conducted the analysis and wrote the initial draft of the paper to which all authors contributed. K.L. and M.A. studied the puma populations in the field and provided the blood samples. S.K., D.R.T., P.S., R.G. and S.V. collected virus and host genetic data. S.D., G.B., M.C. and X.D. contributed to the phylogenetic and transmission tree analyses. M.L.J.G. contributed to the spatial analysis. M.E.C., S.V., K.C., W.C.F. and S.C. conceived of the project.

Competing interests

The authors declare no competing interests.

Animal care statement

Puma samples were collected as part of ongoing studies by CPW between 2006 and 2014. We handled all pumas in accordance with approved CPW ACUC capture and handling protocols (ACUC file no. 08-2004, ACUC protocol nos. 03-2007 and 16-2008). Samples were provided to Colorado State University for diagnostic evaluation. Colorado State University and CPW Institutional Animal Care and Use Committees reviewed and approved this work before initiation (CSU IACUC protocol 05-061A).

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41559-021-01635-5.

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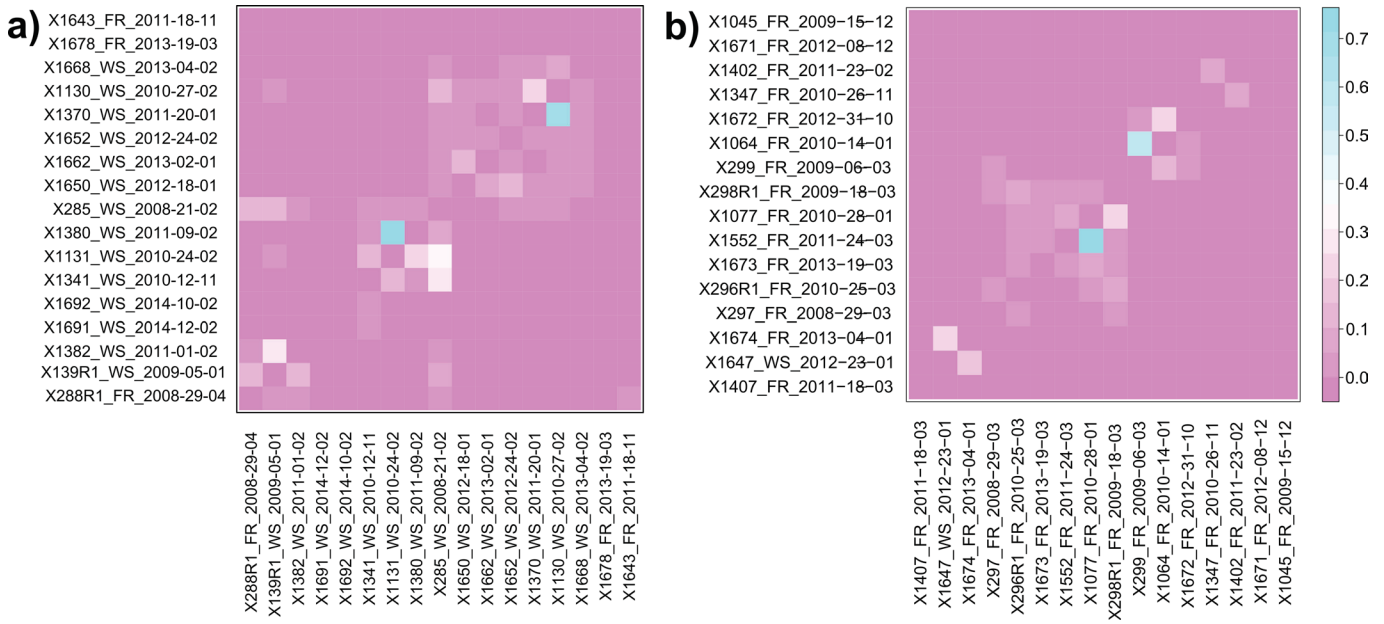
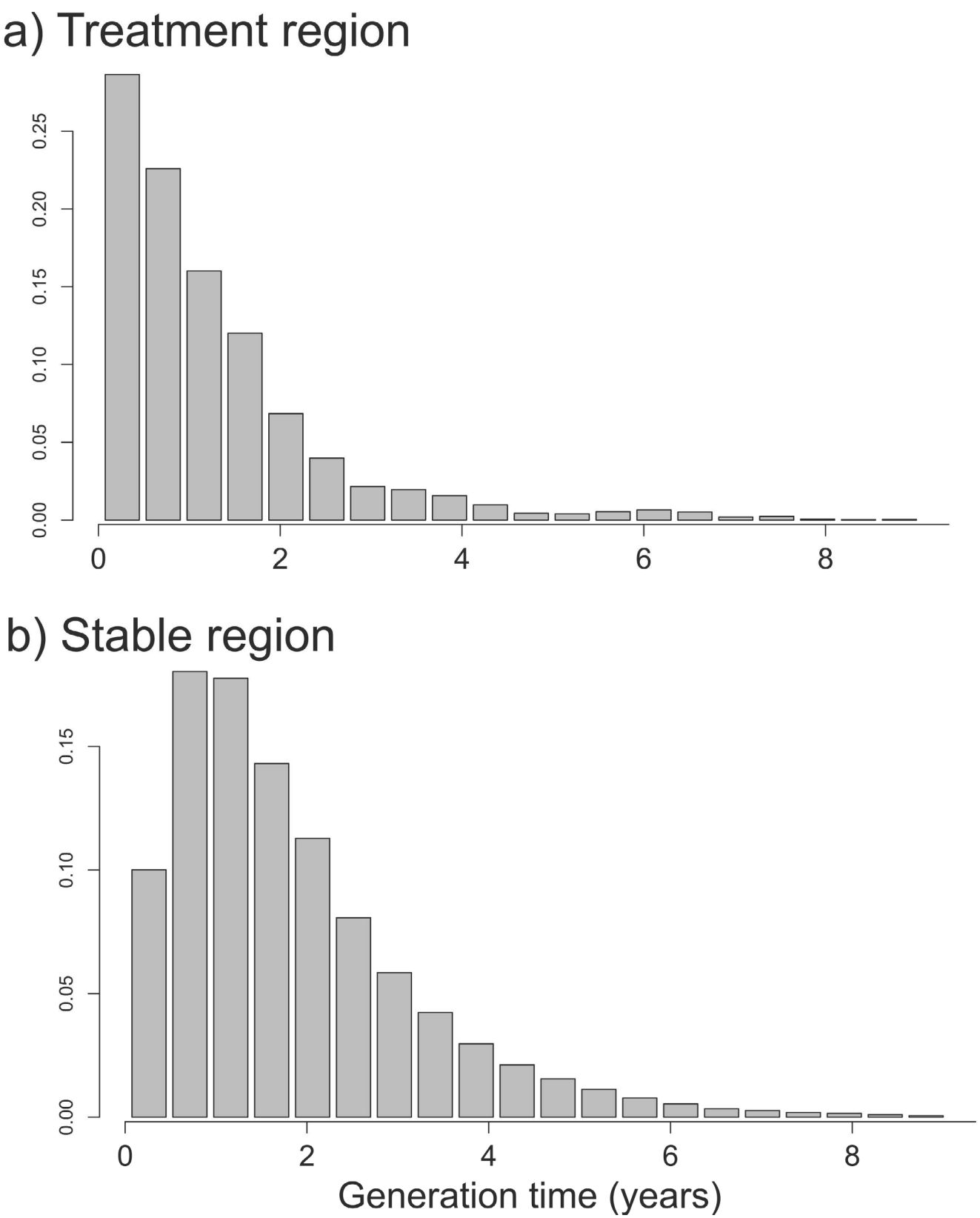
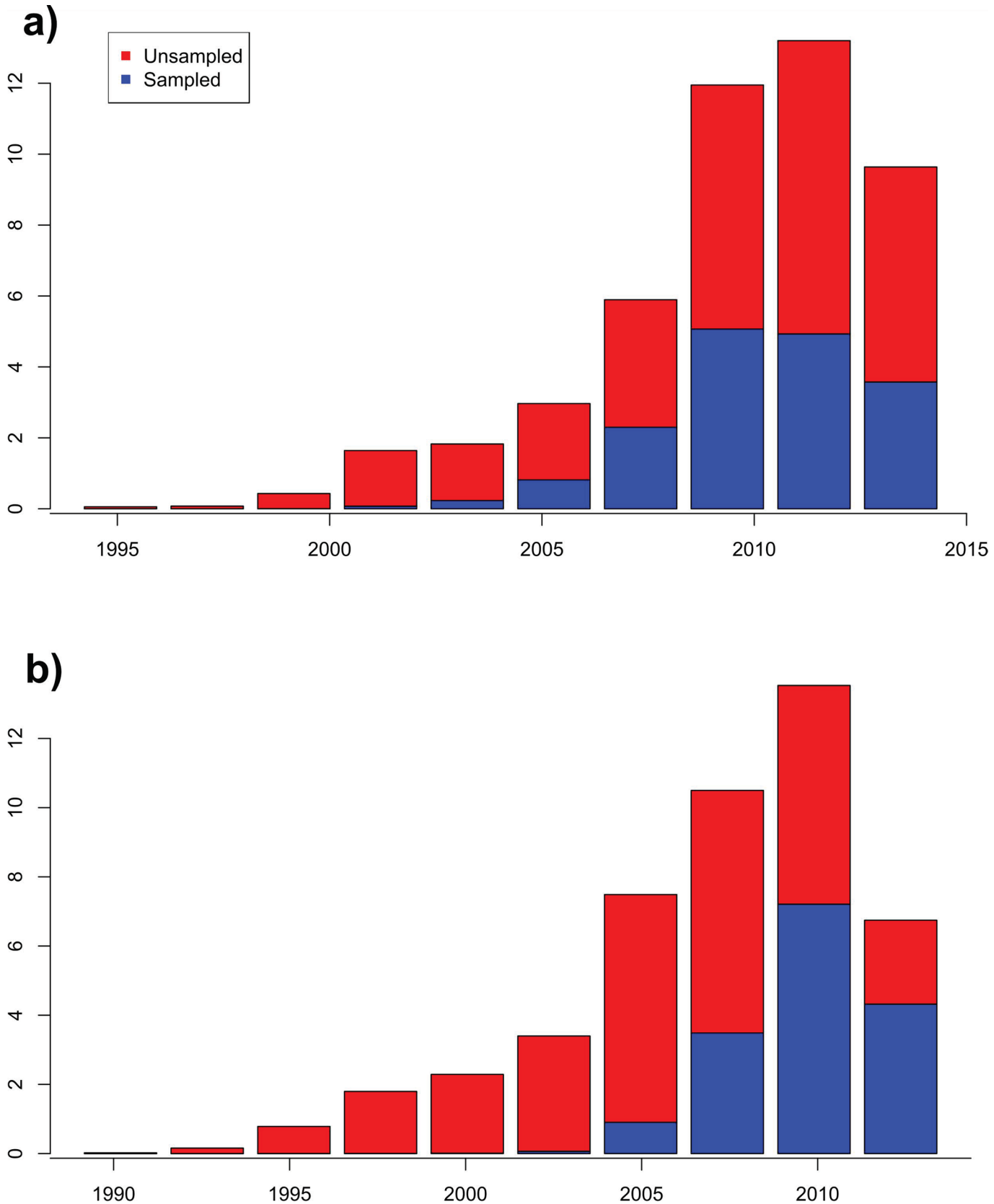


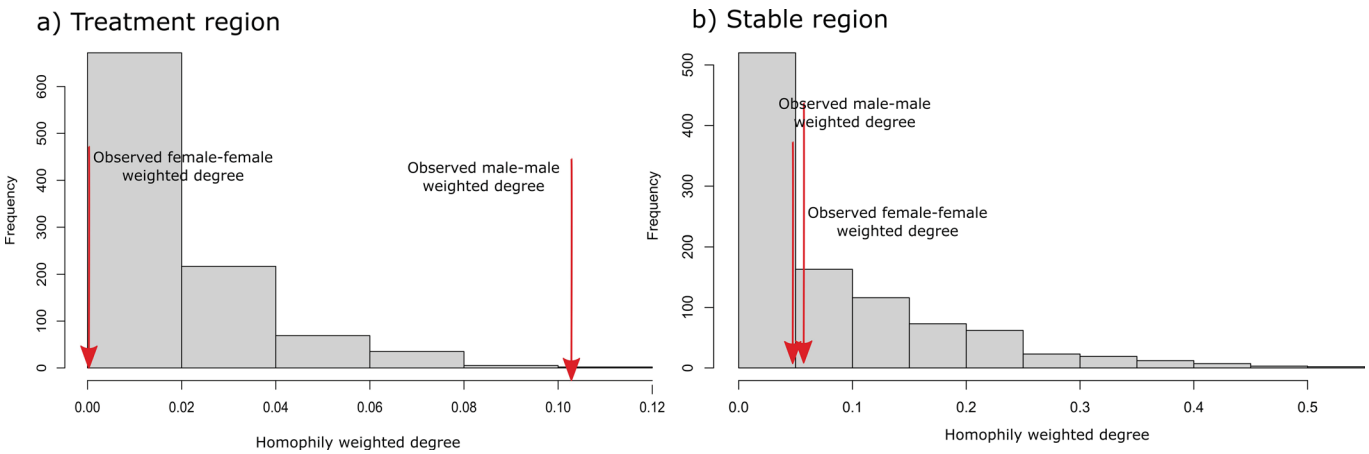
Extended Data Fig. 1 | Probabilities of transmission between pairs of individuals in both regions. The left panel (**a**) shows the treatment region and the right panel (**b**) shows the stable region.



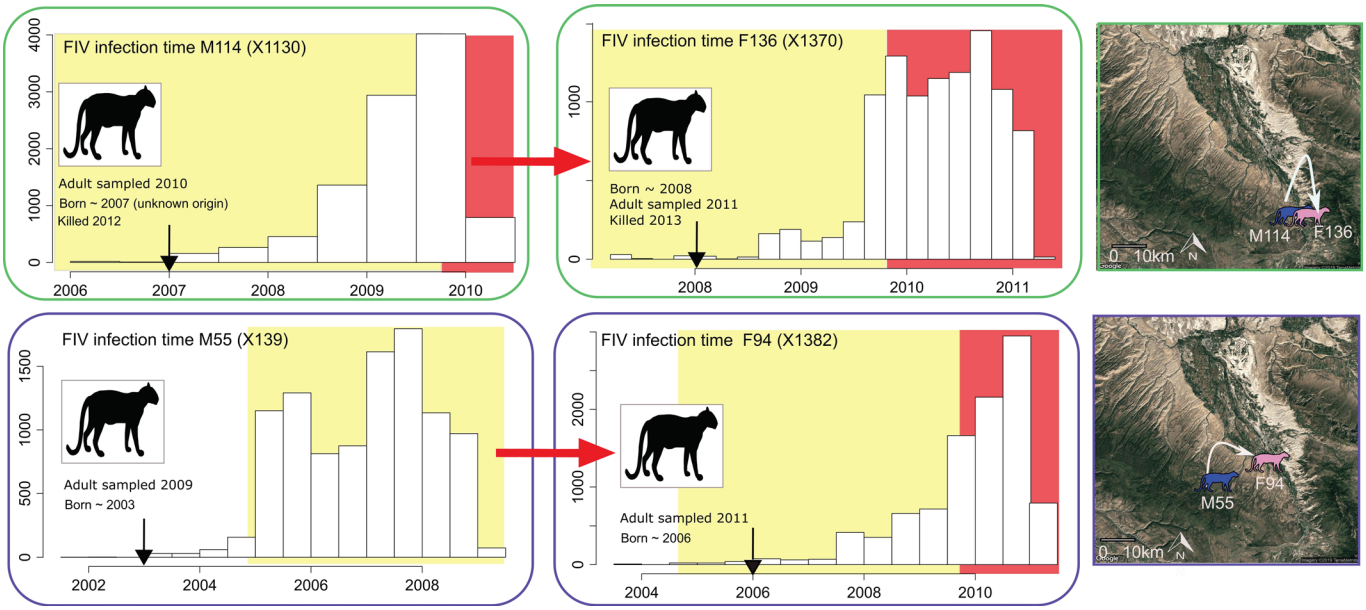
Extended Data Fig. 2 | Realized generation time distributions (time from infection to onward transmission) by region. The top panel (**a**) shows the treatment region and the bottom panel (**b**) shows the stable region. In both regions, onward transmission events for FIVpco were most likely in the first two years after infection.



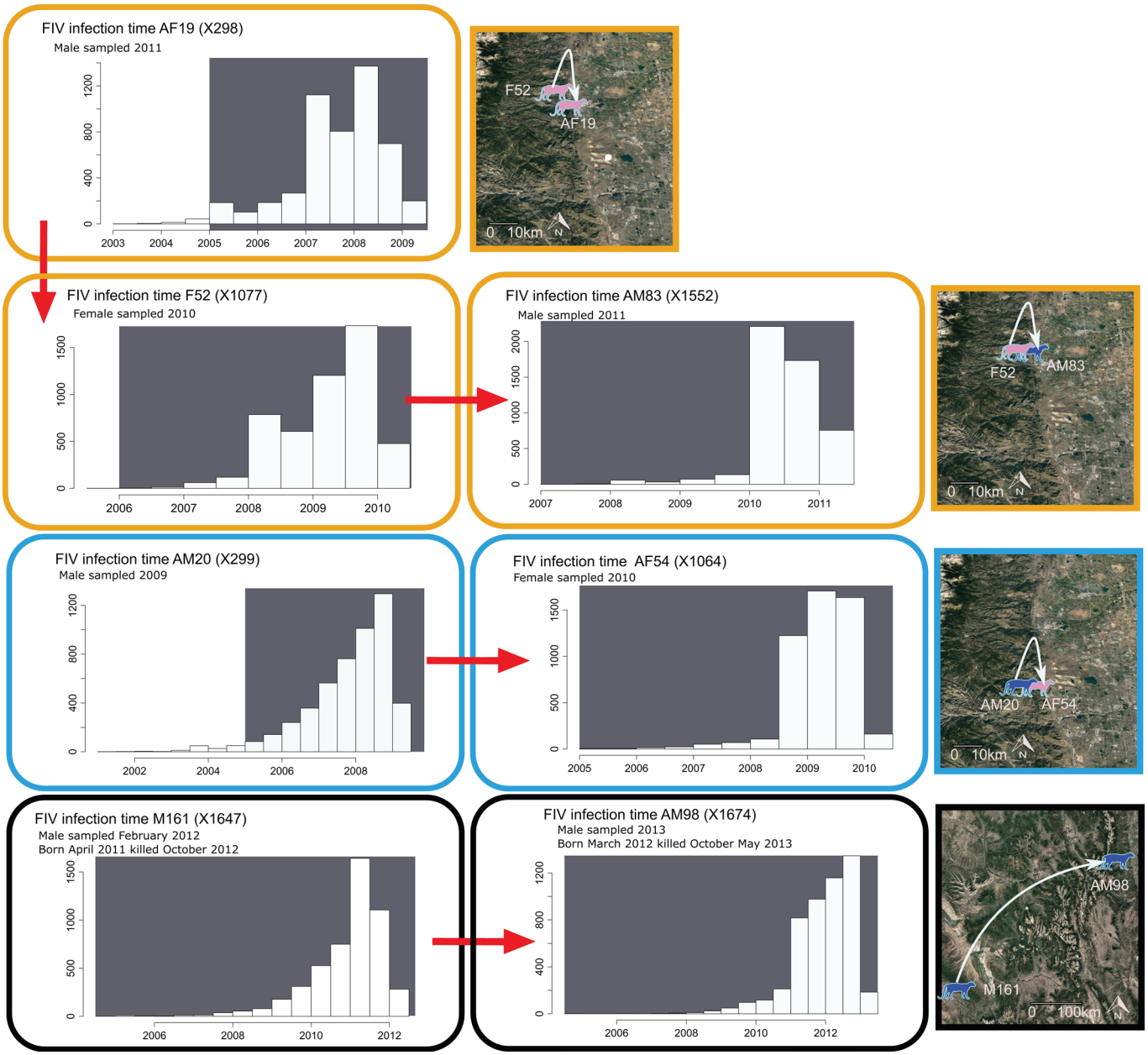
Extended Data Fig. 3 | Estimated number of unsampled vs. sampled cases by region. The top panel (**a**) shows the treatment region and the bottom panel (**b**) shows the stable region.



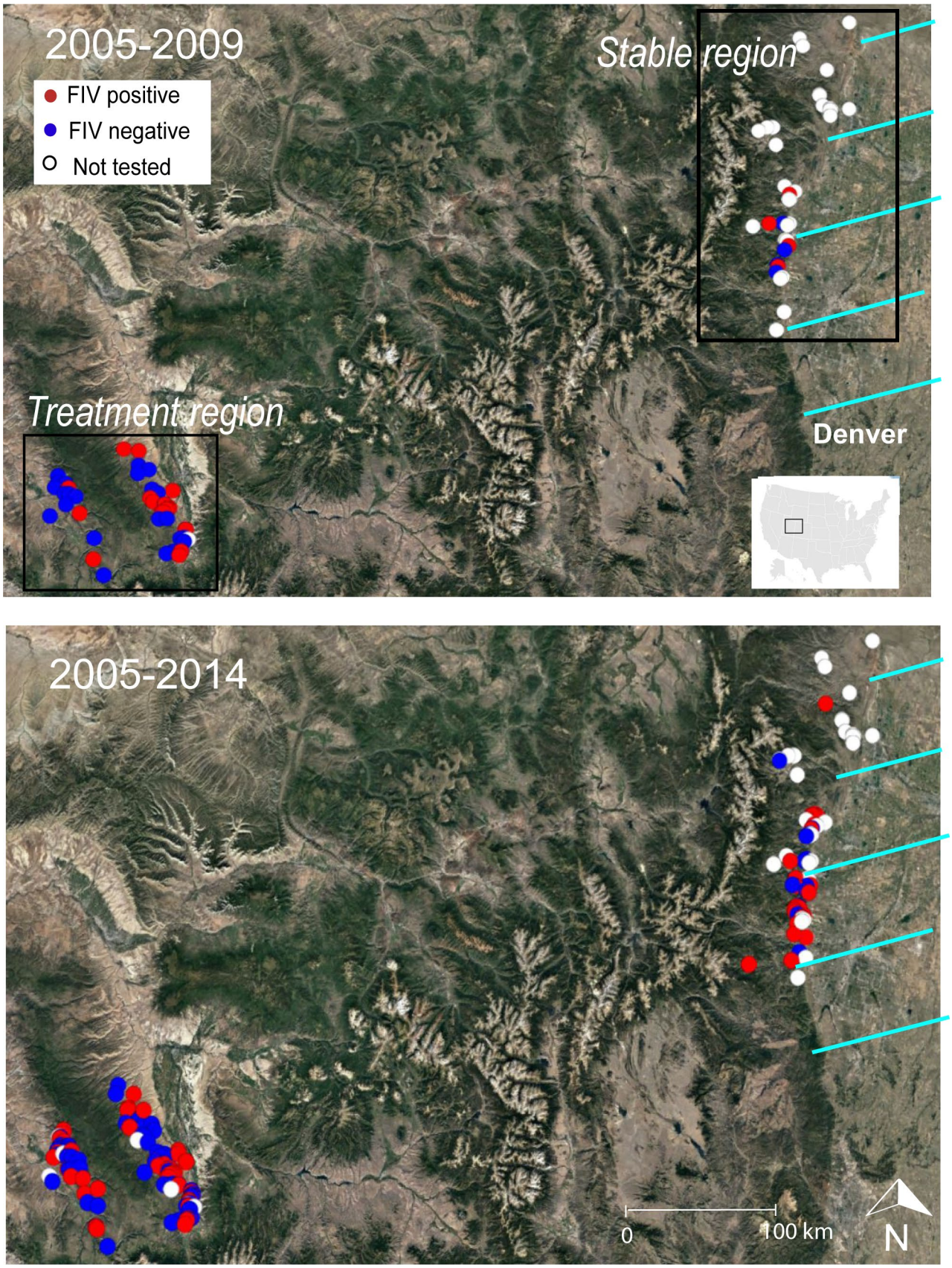
Extended Data Fig. 4 | Histograms showing the expected homophily weighted degree distribution from our simulated networks by region (that is, just including edges from males-males) compared to observed homophily weight degree values. The left panel (**a**) shows the treatment region and the right panel (**b**) shows the stable region. (see Methods -simulation modelling for details).



Extended Data Fig. 5 | Time distributions for individuals involved in a putative transmission chain with the likely direction (red arrows) and the spatial context on each transmission event. See Fig. 2 for other putative transmission events in the treatment region. Light yellow background: hunting pressure relieved, red background: hunting pressure resumed. White arrows in the maps indicate the likely transmission direction. Birth, death and sampling date is provided under each silhouette and estimated birth year is indicated by the black arrow. Colour of the boxes reflects transmission chain identity. M114 and F136 had overlapping home ranges and potentially transmitted FIV_{pco} during mating event(s). M55 and F94 also had overlapping home ranges, and M55 was likely the sire of F94's kittens; the pair consorted on 15 April 2010 and kittens were born on 15 July 2010. In addition, M55 associated with this family when the kittens were nurslings (K. Logan observation). Maps Data: Google ©2020.

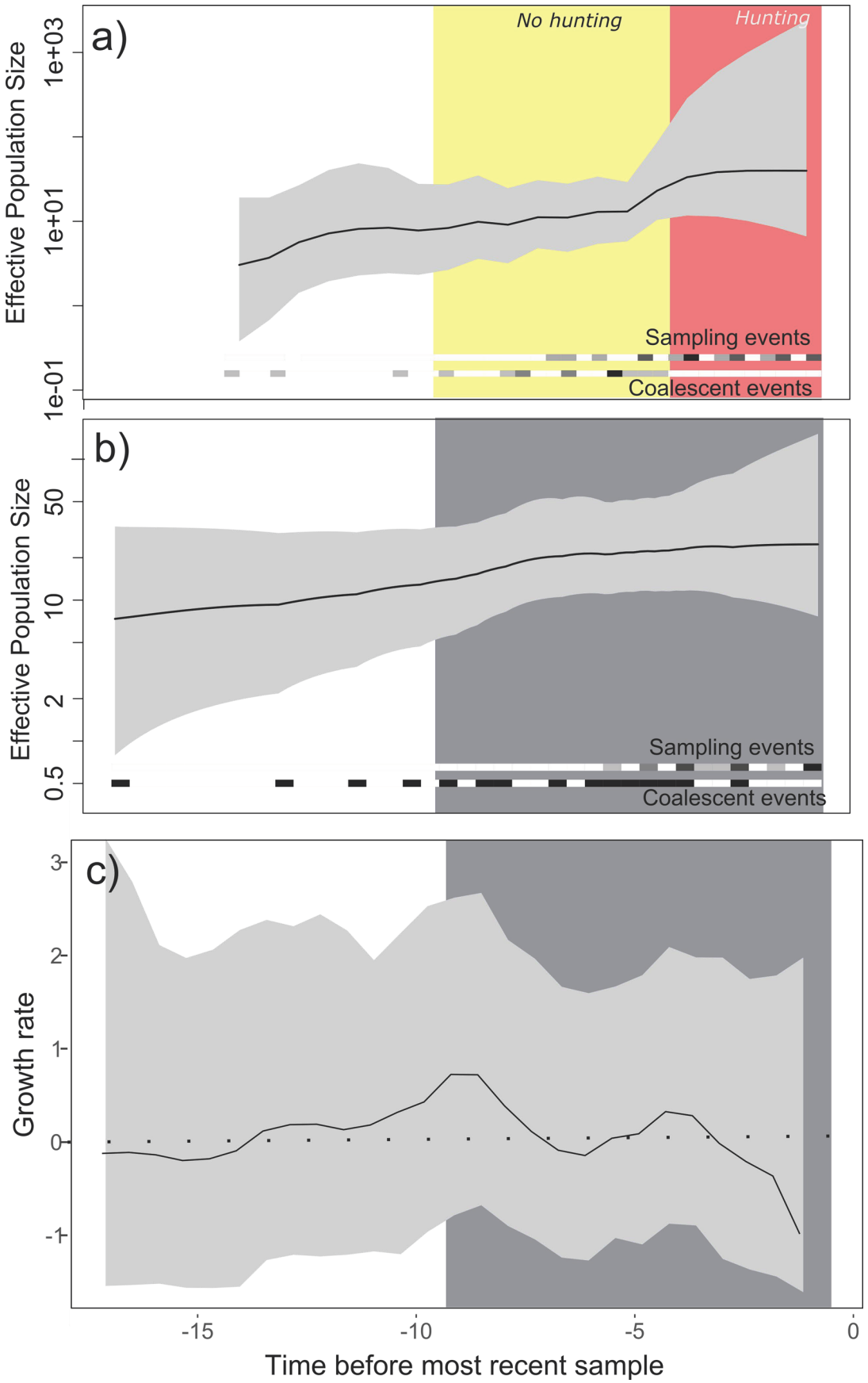


Extended Data Fig. 6 | Infection time distributions in the stable region for individuals involved in a putative transmission chain with the likely direction (red arrows) and the spatial context on each transmission event. Colour of the boxes reflects transmission chain identity and grey boxes indicates sampling period. Sex, sampling date, birth date and death date of each individual are provided in each box when known. Maps Data: Google ©2020.



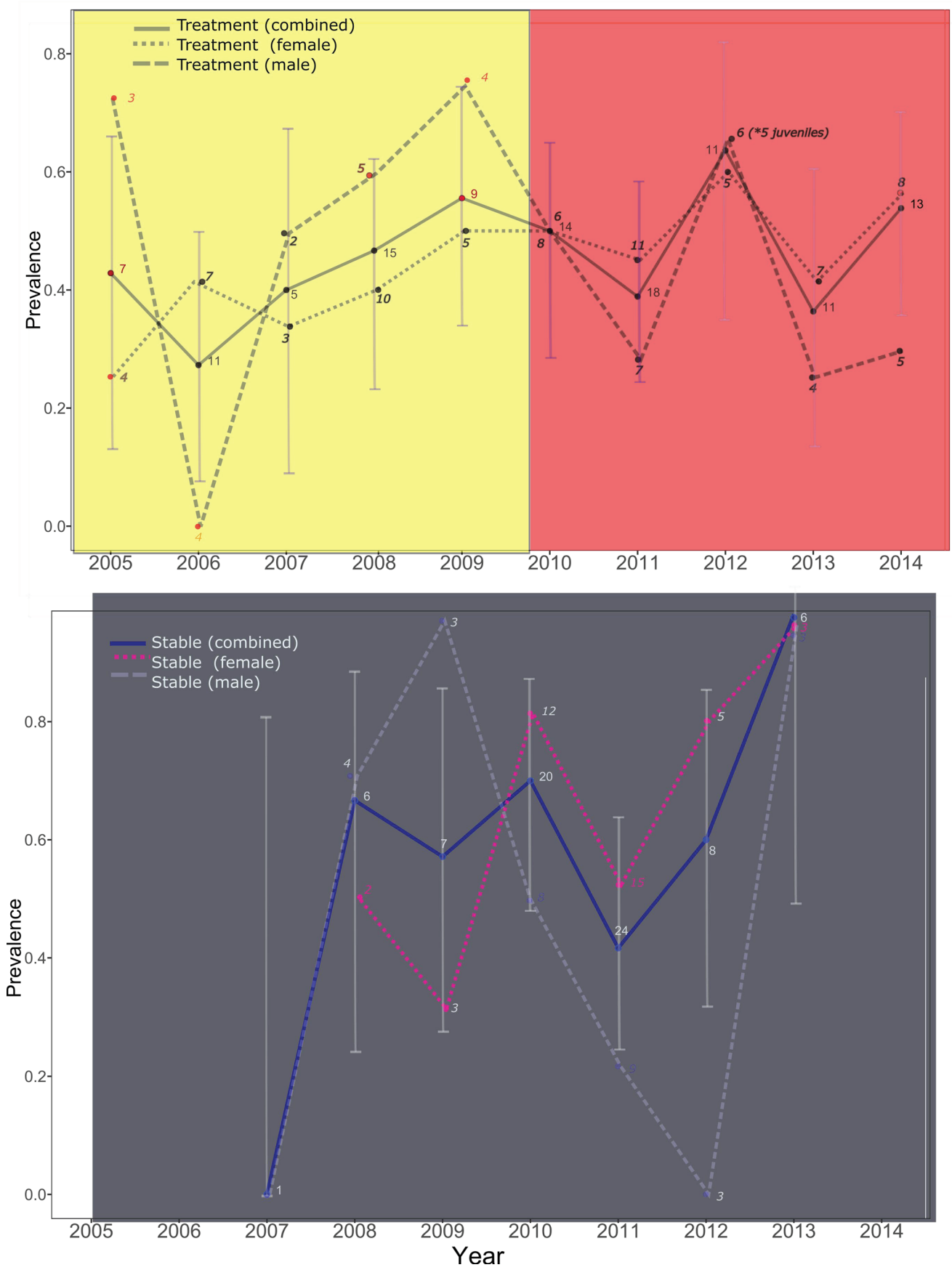
Extended Data Fig. 7 | See next page for caption.

Extended Data Fig. 7 | Map of our study regions. Top panel: the location of all individuals sampled in 2005–2009 (no hunting in the treatment region). Bottom panel: the location of all individuals sampled (2005–2014 including the years when hunting was resumed in the treatment region). White diagonal lines show the broad extent of the Denver metropolitan area. Maps Data: Google ©2020.



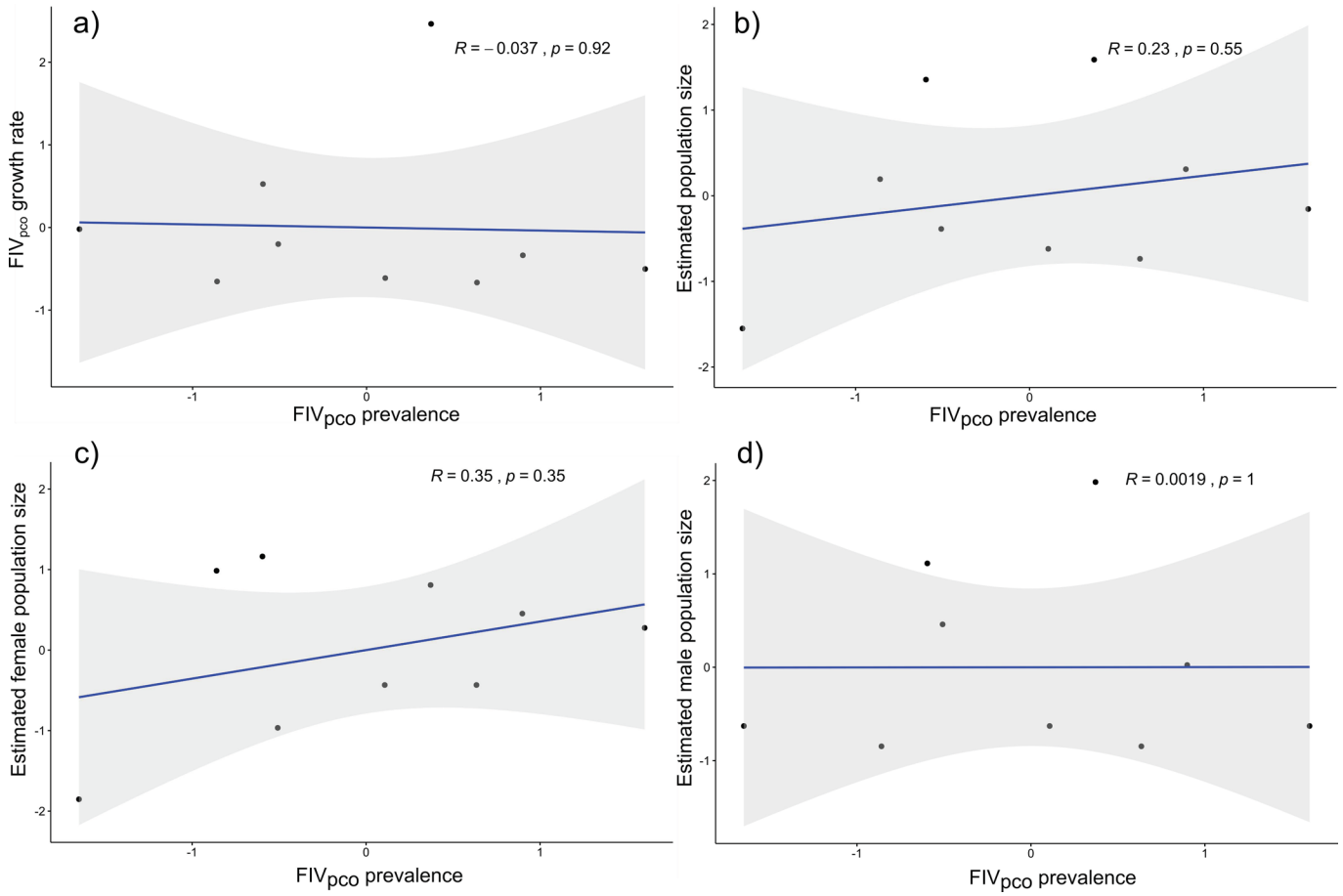
Extended Data Fig. 8 | See next page for caption.

Extended Data Fig. 8 | Skyline plots showing the effective population size through time of dominant FIV_{pco} lineages in each region. The top panel (**a**) shows the treatment region and the bottom panel (**b**) shows the stable region. Grey shading provides the 95% high posterior density (HPD) estimates. a) Light yellow: hunting pressure relieved, red: hunting period, grey background: stable region. **c**) skygrowth plot from the management stable region showing FIVpco growth rate through time (see Fig. 3b in the main text for the corresponding plot from the treatment region). The dashed horizontal line reflects the 0-growth line.



Extended Data Fig. 9 | See next page for caption.

Extended Data Fig. 9 | FIV_{pco} prevalence through time for each region. The top panel (a) shows the treatment region and the bottom panel (b) shows the stable region. Numbers next to the points indicate how many samples were screened using qPCR each year. Confidence intervals were calculated using a binomial distribution and are only shown for total population estimates rather than for each sex (to aid interpretation). **a**) Light yellow: hunting pressure relieved, red: hunting period.



Extended Data Fig. 10 | There were only insignificant relationships between FIV_{pco} prevalence, growth rate and population size estimates. (**a**) FIV_{pco} growth rate (**b**) estimated puma population size, (**c**) female and (**d**) male population sizes in the treatment region. All data are scaled. Similar data was not available for the stable region. R: R2.

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☐	☒ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No software was used
Data analysis	Data analysis was performed in R and code is available here: https://github.com/nfj1380/Transmission-dynamics_huntingPumaFIV

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

DNA sequences—GenBank accession: MN563193 - MN563239. All other data and code to perform the analysis are available on Github: https://github.com/nfj1380/Transmission-dynamics_huntingPumaFIV68

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	This study is based off sequences collected from 213 puma sampled in two regions of comparable size with differing levels of anthropogenic development from 2003-2014.
Research sample	The research sample represented an indiivdual puma (Puma concolor).
Sampling strategy	Our sampling effort represents a large proportion of all individuals in each region (~80%). As puma are solitary big cats, sampling individuals in the wild is logistically challenging.
Data collection	In each region, puma were trapped and anesthetized and blood samples collected according to Colorado Parks and Wildlife protocol. Blood samples were taken back to the laboratory and tested for FIV using quantitative PCR. DNA extraction and sequencing was performed on FIV positive samples.
Timing and spatial scale	The data was collected from 2003-2015 over two ~3000 km2 regions in Colorado.
Data exclusions	For four individuals, the sequencing data was of very poor quality (high proportion of missing sites) and these were not included.
Reproducibility	Our findings are based off observations from two regions and puma in these regions could be resampled.
Randomization	Our study compared observations in two regions so randomization is difficult.
Blinding	Blinding is not relevant to the study.

Did the study involve field work? ☒ Yes ☐ No

Field work, collection and transport

Field conditions	Most blood samples were taken during winter when puma tracks are visible.
Location	Rocky Mountains, Colorado
Access & import/export	Samples were taken by trained professionals from Colorado Parks and Wildlife
Disturbance	Animals were relased post-anesthesia.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	*For laboratory animals, report species, strain, sex and age OR state that the study did not involve laboratory animals.*
Wild animals	Puma blood and tissue samples were collected from 213 individuals (91 males, 109 females).
Field-collected samples	*For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.*
Ethics oversight	Ethics approval attained from Colorado Parks and Wildlife as trapping occurred as part of their population management responsibilities.

Note that full information on the approval of the study protocol must also be provided in the manuscript.