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Title: The contribution of epidemiological predictors in unravelling the phylogeographic history of HIV-1 subtype C in Brazil

Running title: The phylogeography of HIV-1C in Brazil

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Abstract

The phylogeographic history of the Brazilian HIV-1 subtype C (HIV-1C) epidemic is still unclear. Previous studies have mainly focused on the capital cities of Brazilian federal states and the fact that HIV-1C infections increase at a higher rate than subtype B in Brazil calls for a better understanding of the process of spatial spread. A comprehensive sequence dataset sampled across 22 Brazilian locations was assembled and analyzed. A Bayesian phylogeographic generalized linear model approach was used to reconstruct the spatiotemporal history of HIV-1C in Brazil considering several potential explanatory predictors of the viral diffusion process. Analyses were performed on several subsampled datasets in order to mitigate potential sample biases. We reveal a central role for Porto Alegre city, the capital of the southernmost state, in the Brazilian HIV-1C epidemic (HIV-1C_BR), and the northwards expansion of HIV-1C_BR could be linked to source populations with higher HIV-1 burden and larger proportions of HIV-1C infections. The results presented here bring new insights to the continuing discussion about the HIV-1C epidemic in Brazil, and raise an alternative hypothesis for its spatiotemporal history. The current work also highlights how sampling bias can confound phylogeographic analyses and demonstrates the importance of incorporating external information to protect against this.

Importance: Subtype C is responsible for the largest HIV infection burden worldwide, but our understanding of its transmission dynamics remains incomplete. Brazil witnessed a relatively recent introduction of HIV-1C compared to HIV-1B, but it swiftly spread throughout the South, where it now circulates as the dominant variant. The northward spread is comparatively slower and HIV-1B still prevails in this region. While epidemiological data and viral genetic analyses have both independently shed light on the dynamics of spread in isolation, their combination has not yet been explored. Here, we complement publically available sequences and new genetic data from 13 cities with epidemiological data to reconstruct the history of HIV-1C spread in Brazil. The combined approach results in more robust reconstructions and can protect against sampling bias. We found evidence for an alternative view on the HIV-1C spatiotemporal history in Brazil, which, contrary to previous explanations, integrates seamlessly with other observational data.

Key Words: Brazil; HIV-1 subtype C; phylogeography; generalized linear models; epidemiological predictors.

Introduction

Upon emergence into the human population in central Africa around 1920 (1), HIV-1 group M has diversified into nine subtypes and numerous circulating recombinant forms (CRFs) through a series of founder effects and recombination events (2,3). Although HIV-1 subtype B (HIV-1B) dominates in many countries in Europe and Americas (2), more than 50% of the infections worldwide are caused by HIV-1 subtype C (HIV-1C), which is the most prevalent subtype in southern Africa countries and India, and is increasing in prevalence in China and South America (2,4).

The epidemic in Brazil is mainly driven by HIV-1B, followed by lower frequencies of HIV-1C, F1 and BF1 recombinants (5). In the southern regions of Brazil, however, the spread of HIV-1B is matched by HIV-1C, which co-circulates in similar proportions and can even be responsible for up to 80% of the infections (4). In addition, the two southernmost Brazilian states, Rio Grande do Sul (RS) and Santa Catarina (SC), have the highest AIDS incidence in the country (6). These patterns have motivated several investigations into the history and dynamics of the Brazilian HIV-1C epidemic (HIV-1C_BR), which estimated an origin in the 1960s-1970s in the state of Paraná (PR) (7,8,9). Because the HIV-1C incidence in more northern states has only recently begun to increase (4,10-14), this suggests viral diffusion would be driven by unknown factors that promote a fast dissemination to the south while constraining spread to the north.

HIV infections are characterized by a dynamic viral population of closely related variants that can quickly adapt to changing selective pressures, which manifests in a formidable speed at which genetic diversity accumulates within hosts (15). This rapid accumulation of genetic diversity makes HIV-1 a prime example of ‘measurably evolving populations’ (16). As a consequence, there has been an important role for phylogenetic tools to shed light on the epidemiological history of HIV. In fact, this has stimulated many developments in the field of statistical phylodynamics, such as molecular clock models to incorporate sampling time as calibration information (17, 18) and coalescent models to infer the changes in viral population size over time (19-21). More recently, such genealogy-based population genetic inferences have also been complemented with state-of-the-art phylogeographic tools (22-24). Phylodynamic analyses of HIV-1 have culminated in a relatively rich statistical account of its evolutionary and epidemiological history (1).

While these statistical models and inference tools have proven invaluable for testing hypotheses using virus genetic data (25, 26), they are limited in their ability to link epidemic processes to pathogen evolution because non-genetic data is usually not directly incorporated into the models. For phylogeography, this has recently been addressed by extending a Bayesian discrete phylogenetic diffusion approach in order to incorporate covariates in the process of spread (27). This approach estimates phylogeographic history while identifying the contribution of several potential explanatory variables (predictors) of spatial spread and allows for cross-talk between the spatial genetic distribution and the relevant predictors: the predictors are selected for the ability to explain the location transition history, but by helping to inform the process parameters they can also assist in shaping the ancestral reconstructions. This approach has already proven useful to elucidate the drivers of both human and swine influenza dispersal (27, 28).

In the present study we reconstruct the phylogeographic history of HIV-1C in Brazil incorporating newly obtained sequence data. While previous studies mostly included sequences from state capital cities, we here expanded the spatial sampling by including HIV-1C sequences from 10 rural locations in the southernmost states RS and SC. Our study demonstrates for the first time that augmenting the molecular sequence data with relevant epidemiological information can contribute to the robustness of phylogeographic reconstructions.

Methods

Patients, samples and new sequences:

A total of 360 HIV-1 seropositive patients from 13 cities from the states of SC and RS (Figure 1) were enrolled in this study, which was approved by the ethics committees of the Federal University of Santa Catarina and the Foundation of Research and Production in Health of the Rio Grande do Sul state. Between October 2009 and February 2014 blood samples were collected and HIV-1 envelope (HXB2 6846-7360 bp) and polymerase (HXB2 2274-3545 bp) fragments were amplified from whole cellular DNA by nested-PCR and sequenced as described elsewhere (29). Sequences were subtyped using the REGA, RIP and SCUEAL online subtyping tools (30-32) and by performing maximum likelihood phylogenetic inference incorporating HIV-1 subtype reference sequences available from the Los Alamos HIV sequence database (www.hiv.lanl.gov). Recombinant sequences were identified through bootscanning analysis using Simplot 3.5.1 (33) (see Supplementary Text S1, for methodological details). The sequences generated in the present study were deposited in GenBank under accession numbers: KR065788-KR066336 and KP224476-KP224501.

Sequence dataset compilation:

Briefly, we complemented our new sequence data with all publically available Brazilian HIV-1C sequences (HIV-1C_BR) from *pol* and *env* genes (*n* = 385). Non-Brazilian HIV-1C sequences were selected using BLAST+ (34). To this purpose, we created a local BLAST database that contained all HIV1-C sequences minus those from Brazil. We performed a similarity search on this database using every HIV-1C_BR sequence as a query, and the 50 best hits for each search were logged. Duplicate entries were removed from these hits and compiled as the international database. After extensive data cleaning this resulted in datasets with 1522 *pol* and 621 *env* sequences that were down-sampled to around 500 sequences each to reduce the computational burden in subsequent Bayesian statistical analyses (see Supplementary Text S1, for full details of the followed procedure). Six additional sub-samplings containing only Brazilian sequences were made for *pol* and *env*, to allow assessing the robustness of the phylogeographic reconstructions (see below). For this we aimed at reducing sampling bias by creating three random down-samples in two groups: a) Rand10 - with a maximum of ten sequences by location and b) Rand20 - with a maximum number of 20 sequences by location (see Supplementary Table S1, for HIV-1C_BR sequences in each dataset).

Phylogenetic divergence time estimation and population dynamics inference

Time-scaled phylogenetic trees were reconstructed using a Bayesian inference method implemented in the BEAST/BEAGLE software (35,36). All analyses were performed using the GTR+I+Γ_4 nucleotide substitution model and an uncorrelated lognormal relaxed molecular clock under the Bayesian Skyride coalescent model (18,37). Due to the low temporal signal of the datasets, the use of an informative prior on the tMRCA of the Brazilian subype C clade was required. For this purpose, we specified a normal distribution with mean (1976) and standard deviation (5.1) based on previous estimates of the time of introduction of subtype C in Brazil (8). When exact sampling dates were unknown, the dates were integrated out over a known sampling time interval (38). Monte Carlo Markov Chains (MCMC) were run sufficiently long to ensure stationarity and adequate effective sample size (ESS >200) as diagnosed by Tracer (http:// beastbioedacuk/Tracer). Maximum clade credibility (MCC) trees were summarized using the TreeAnnotator tool and visualized in Figtree v1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/). A representative sample of 1000 trees was collected and used as an empirical tree distribution for estimating the virus migration processes (see below). To ensure that subsequent phylogeographic analyses are based on histories specific to Brazil we pruned sequences clustering outside the HIV-1C_BR cluster or non-Brazilian sequences clustering inside HIV-1C_BR cluster from these trees using PAUP (http:// http://paup.csit.fsu.edu) (see Text S1, for methodological details).

Phylogeny-trait association:

We tested for significant phylogenetic clustering by location in two different ways. First, we calculated the Association Index (AI), Parsimony Score (PS) and Monophyletic Clade (MC) measures using BaTS (39). For our second approach we introduced the use of the path sampling (PS) and stepping-stone (SS) sampling marginal likelihood estimators as implemented in BEAST (40,41) to evaluate the extent to which the topology is required as a correlation structure to explain the traits. For this we specified a discrete symmetric (reversible) model of location transitioning and incorporate Bayesian stochastic search variable selection (BSSVS) procedure (22) fitting the trait diffusion process on: 1) a fixed MCC tree summarized from the Bayesian phylogenetic analysis of the complete dataset; 2) a star-like tree with the same trait annotations as in the MCC tree.

Phylogeographic inference with epidemiological predictors

To assess the impact of potential explanatory variables (predictors) of the viral diffusion process on phylogeographic reconstructions, we made use of the recent generalized linear model (GLM) extension of Bayesian discrete phylogeographic models (27). This allows reconstructing the spatial diffusion history throughout the tree while simultaneously evaluating the contribution of various potential predictors. Support for predictors is estimated using a BSSVS procedure, and the contribution of each predictor is quantified as a GLM coefficient that has an impact (effect size) in the transition rate among the locations.

Using this approach, we tested the following predictors (see Text, SDC 1, for methodological details):

1) Geographic distance: the great-circle distance among each pair of cities;

2) Passenger air traffic: the number of passengers traveling between each pair of airports;

3) HIV population size: the total number of AIDS notifications in a period of 10 years reported in each city;

4) HIV prevalence: (HIV population size / city population size) X 100,000 habitants;

5) HIV-1C population size: HIV population size times the proportion of HIV-1C as reported in the literature (4,10-14);

6) HIV-1C prevalence: (HIV-1C population size / city population size) X 100,000 habitants;

7) Sample size: the number of sequences by location.

Because not all sampling locations have an airport, we specified a different geographic partitioning for evaluating predictor 2 (passenger air traffic). This partitioning is not well suited for the epidemiological predictors, which led us to test predictor 2 in separate analyses including only sample size as an additional potential predictor.

GLM analyses were run in BEAST using previously recommended prior specifications on the set of empirical trees obtained by the Bayesian phylogenetic analysis (27). Bayes Factors (BF) were calculated to determine the support for the inclusion of each predictor in the model and predictor contributions are reported as effect sizes conditional on the effect being included in the model.

A phylogeographic analysis with BSSVS was performed with asymmetric transition rates being informed by the predictors supported by the GLM analysis. In other words, for each sub-sampled dataset, we used the rate estimates for prior specification based on the corresponding GLM analysis. SPREAD software was used to identify the well-supported transition rates based on BFs > 3 (42). We complemented this analysis with Markov jump estimation of the number of location transitions throughout the evolutionary history (43). RStudio (http://www.rstudio.org/) was used to calculate the Bayes factors and effect sizes, and to summarize the posterior densities of the highly supported transitions from the BEAST log files.

Results

Sequence dataset compilation

We sequenced 140 *pol* and 202 *env* HIV-1C isolates in 13 locations in SC and RS states, 10 of which have not been sampled before (Figure 1). By combining the generated sequence data with publicly available Brazilian and international HIV-1C sequences we were able to compile comprehensive *pol* and *env* based datasets for reconstructing the spatiotemporal history of HIV-1C in Brazil. In summary, the complete *pol* dataset contained 380 Brazilian and 120 international sequences while the *env* dataset totaled 293 Brazilian and 170 international sequences (see Supplemental Dataset 1, for complementary information about sequences retrieved from public databanks). The Brazilian *pol* sequences are distributed over 21 locations and the *env* sequences represent 17 locations totalizing 22 locations represented with *pol* or *env* sequences, most of them in SC and RS (15/21 for *pol* and 14/17 for *env*). Considering the complete Brazilian dataset, sequences represent the time period between 2002 and 2014 (see Supplementary Table S1).

Phylogeny-trait association

Because our preliminary analyses suggested a considerable degree of phylogenetic mixing by location, we formally tested whether the datasets containing only Brazilian sequences still supported spatial population structure. The hypothesis of a panmictic population could be rejected for the *pol* and *env* datasets by the association index (AI) and parsimony score (PS) statistics (p<0.05), but the monophyletic clade (MC) scores revealed that for 12/21 (57%) of *pol* locations and 12/17 (71%) of *env* locations random clustering could not be rejected (see Supplementary Table S2, for MC scores). The results of the approach based on model testing also provided strong support against the absence of phylogenetic association by sampling location in the *pol* and *env* datasets (Bayes factors of 74 and 39 respectively).

Inconsistencies in root state estimates

The results of the phylogeographic reconstruction showed, with strong agreement between most datasets and models applied (50/56 analyses), that the epidemic ignited in SC or RS. Its exact location of introduction could, however, not be unambiguously determined using only virus genetic data. Whereas in the complete *pol* and *env* datasets Florianópolis (FLP) was consistently estimated as the most likely location at the root, other cities - most notably Porto Alegre (POA) (7/48) and Criciúma (CRI) (7/48) - were implicated in 60% (29/48) of the analyses based on the Rand10 and Rand20 subsampled datasets (Table 1).

Predictors of viral spread

Using a phylogeographic GLM approach, we evaluated which measures predict the rates of location exchange in the complete and subsampled datasets (Table 2). In the *pol* and *env* complete datasets only the origin and destination sample size yielded strong Bayes factor (BF) support, reflecting the fact that only sample sizes and their heterogeneity are needed to explain the number of location transitions. We also found strong support for destination sample size in the model as a predictor in all *pol* and *env* subsampled datasets (Rand10 and Rand20). This indicates that despite the more homogeneous distribution of sequences by sampling locations in these subsampled datasets, the remaining heterogeneity still has an impact on the phylogeographic reconstructions.

Two predictors, “origin HIV prevalence” and “origin subtype C population size”, were included in all *pol* and *env* Rand10 and Rand20 datasets with Bayes factor estimates ranging from moderate (BF=6) to strong (BF=39) support and with positive mean conditional effect sizes (Figure 2). Hence, locations with higher HIV prevalence and larger HIV-1C populations tend to act as sources for onwards spread.

In addition to epidemiological predictors we also tested geographical distance or air transportation data (in a separate analysis, data not shown) as a predictors of HIV-1C diffusion, but these did not result in noticeable support by any of the analyzed datasets.

Interestingly, incorporating relevant epidemiological information into the phylogeographic reconstructions resulted in consistent root state estimates: using the GLM model we find POA as the modal root state in all (12/12) *pol* and *env* Rand10 and Rand20 datasets. Only in the complete datasets, where the sampling bias is more severe, Florianópolis (FLP) was still estimated as the modal root state.

To assess the robustness of the phylogeographic reconstructions with respect to the root height prior (see Methods), we also performed the ancestral reconstruction using genealogies estimated under priors that specified a mean tMRCA that was 10 years older and younger respectively. We find that differences in tree depths did not impact the outcome: POA is consistently the modal root state and the same predictors find substantial Bayes factor support in all *pol* and *env* Rand10 and Rand20 datasets of the extended and shortened histories.

Porto Alegre as a central hub of the HIV-1C epidemic

We subsequently estimated the most likely migration patterns using an asymmetrical phylogeographic analysis with BSSVS and priors on the location exchange rate priors that are based on the GLM rate estimates. The robustness of the ancestral reconstructions was somewhat lower because in this analysis the predictors can only influence the analysis through the prior specification: POA was found to be the root state location in 10/12 *pol* and *env* Rand10 and Rand20 datasets (data not shown). Nonetheless, POA was strongly linked to all other locations (Bayes factors ≥ 3) while only a few additional well-supported transitions were found. Because this suggests a central role for POA in the Brazilian HIV-1C dissemination, we address its role in more detail.

The arrival of HIV-1C in POA was estimated in 1973 (1966 – 1980, 95% HPD) for *pol* and 1971 (1963-1978, 95%HPD) for *env*, and the spread to other cities started around 1980. The timing of these events reveals a consistent pattern. Nearby locations within RS (Rio Grande and Uruguaiana cities) were initially affected, followed by export to south and southeast state capitals in the early 1980s (e.g. to Florianópolis, Curitiba, Rio de Janeiro and São Paulo). More distant locations were affected at a later stage, first in Central-West region (Campo Grande) in middle 1980s and later in North region (Palmas and Manaus) in the late 1980s and early 1990s. Only two exceptions to this pattern are found (one in the *pol* and one in the *env* datasets): the capital city Goiânia, where HIV-1C appears to have been introduced from POA in 1981 (*pol*), and Rio de Janeiro, where the introduction of HIV-1C has been more recent according to the *env* datasets (see Supplementary Table S3, for time of first transition from Porto Alegre).

More insights into the temporal pattern of spread were obtained by mapping the density of location transitions from POA to the other state capital cities through time. This reveals a period of higher density of viral influx 25 to 30 years ago to the South region capital cities Florianópolis and Curitiba. Among the sampled capitals in the Southeast region, a similar pattern emerged for Rio de Janeiro but there is a more evenly distributed transition density through time to São Paulo. Such a shift of transition density towards more recent times is slightly noticeable for the capital cities of the Central-West and North regions (see Supplementary Figure S1, for transitions by time from Porto Alegre).

Discussion

We reconstructed the phylogeographic history of HIV-1C in Brazil using a comprehensive set of *pol* and *env* subtype C sequences from 22 different cities, of which 10 were sampled for the first time. Using a new model-testing based approach and by calculating several phylogeny-trait association measures using BaTS (39) we could reject random mixing in both datasets. However, as seen in MC scores, not all locations contributed equally to phylogenetic signal resulting in a considerable degree of uncertainty in the phylogeographic inferences. Nevertheless, after balancing the number of samples per location to mitigate the confounding effects of sampling biases, we were able to identify support for two epidemiological predictors of the viral spatial diffusion process.

Specifically, we found higher migration intensity from cities with larger numbers of HIV-1C infected patients and higher HIV prevalence. Interestingly, this is in agreement with a pattern of HIV-1C spread towards the north of Brazil, where the prevalence of HIV is smaller and only few cases of HIV-1C infection are found (4,6). An intriguing result illustrating the complexity of modeling human mobility is that neither "geographical distance" nor "passenger air traffic" predicted viral spread. The sample size of source and/or recipient locations, on the other hand, were always included in the model (in isolation or together, Table 2). Samples sizes are expected to predict the number of transitions to some extent, and it was not our intention to formally demonstrate this. Rather, we wanted to avoid that other predictors would be supported simply because of correlation with sample sizes. In other, words we do not expect the support for HIV prevalence and subtype C population size in the origin locations is artifact of the potential correlation with sample size as this is already accommodated explicitly in the GLM analysis.

To explore how sampling heterogeneity also impacts ancestral reconstructions, we analyzed six random down-sampled datasets in parallel with the complete *pol* and *env* datasets. This highlighted a substantial variability in the root state estimates (Table 1) and confirms that the sampling scheme can indeed have a profound effect on the inferred location state probabilities at the internal nodes of the tree. The impact of sampling biases was most likely aggravated by the relatively high degree of mixing observed in the *pol* and *env* datasets (see Supplementary Table S2, for MC scores). The geographical partitioning is also an important factor in discrete phylogeographic analyses because this determines the level of spatial detail that can be recovered. Whereas previous studies investigating the spread of HIV-1C in Brazil discretized locations according to federal states or geopolitical regions (7-9), we opted for a higher-resolution scheme and defined cities as the locations of interest. This allowed us to include more precise predictors in the GLM model.

We were able to largely resolve sampling-bias related inconsistencies by informing the phylogeographical reconstructions with relevant epidemiological information. Our results consistently identify POA, and not the state of Paraná (7-9), as the point of introduction. Several lines of evidence support this hypothesis. The population in the metropolitan area of POA has about 4 million inhabitants, the largest in the South region, and the AIDS incidence rate in POA and its metropolitan area is the highest rate in Brazil (6). This suggests that the virus found ideal circumstances for transmission and explains why the HIV-1C prevalence in Paraná's capital Curitiba is much lower (~22%) compared to POA (~40% and up to ~60% if the proportion of CRF31_BC - a local circulating form with a small subtype B insertion in a subtype C backbone- is considered) (4, 44).

Differences in risk-group associations between the subtype B and C epidemics in Brazil also seem to support our findings. Whereas in POA the association between men-having-sex-with-men (MSM) and HIV-1B disappeared in more recent sampling because of an expansion of HIV-1C in heterosexual (HET) and MSM groups (45), compartmentalized epidemics are still observed in other cities from the South region, including in Paraná, which could be explained by a later introduction of HIV-1C (4,46-48).

Finally, a central role for POA is also reflected in the high support for transitions from POA to all other locations and the reconstructed temporal pattern of dissemination. After its introduction in the early 1970s, HIV-1C started spreading to other cities in the beginning of the 1980s, first to nearby locations and then to locations progressively further away. It is interesting to note that we could recover a noticeably higher fraction of recent jumps from POA to São Paulo when compared to transitions from POA to other South or South-East region capital cities, which points towards a strong longstanding epidemiological link between both cities.

Although our analysis provides support for POA as the central dissemination point of HIV-1C in Brazil, some caution is required when analyzing the number of transitions in star-like trees such as those typically found for HIV-1. The absence of clear phylogenetic structure deeper in the trees also offers little opportunity to capture clear spatial structuring and transitions beyond those out of the location state at the root. In the current work, our sampling strategy focused on broad geographic coverage rather than on a dense sampling, and a small sample from a large and diverse population that has grown exponentially through time, generally results in star-like topologies. Thus, despite the support for a central role of POA, we can recover little detail on viral spread beyond transitions out of this location.

In conclusion, we present a comprehensive reconstruction of the spatial and temporal dynamics of HIV-1C in Brazil based on *pol* and, for the first time, *env* sequence data, and included data from 10 newly sampled cities. By augmenting the viral genetic information with epidemiological data, we revealed a central role for POA city in the spread of HIV-1C in Brazil. In addition, we also identified locations with high HIV prevalence and large subtype C population sizes as key in the epidemic expansion towards the north of Brazil.

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Figure legends:

Figure 1. Administrative map of Brazil indicating the locations from where HIV-1C sequences were obtained. Pie charts show the HIV-1C (black) percentage of infections relative to other HIV-1 strains (grey) in all cities with *pol* or *env* sequences included in this study. State name abbreviations are shown in bold. The inset shows an enlarged map with the sampling locations (black and red dots) in Santa Catarina and Rio Grande do Sul from which new sequence data were generated. Red dots: cities sampled for the first time. Black dots: sampling locations from where sequence data from other studies were also available. Brazilian regions are colored according to the legend. **Acronyms for states:** AM: Amazonas; GO: Goiás; MS: Mato Grosso do Sul; PR: Paraná; RJ: Rio de Janeiro; SC: Santa Catarina; SP: São Paulo; TO: Tocantins; RS: Rio Grande do Sul. **Acronyms for cities:** BLU: Blumenau; CHA: Chapecó; CPG: Campo Grande; CRA: Cruz Alta; CRI: Criciúma; CTB: Curitiba; CXS: Caxias do Sul; FLP: Florianópolis; GOI: Goiania; ITA: Itajaí; JOI: Joinville; LAJ: Lajeado; LGE: Lages; MAN: Manaus; PAL: Palmas; POA: Porto Alegre; RIG: Rio Grande; RJN: Rio de Janeiro; SPL: São Paulo; STI: Santiago; STL: Santana do Livramento; URU: Uruguaiana.

Figure 2. Significant predictors of the Brazilian HIV-1C epidemic spread among sub-sampling datasets of *pol* and *env* genes. Inclusion probabilities are represented as Bayes factors (BF) and a BF=3 threshold was used as positive indication of the predictor inclusion. The effect of each predictor, conditional to its inclusion, is represented by the posterior mean (black dot) and 95% HPD of the GLM coefficients in log scale.

Table1. Modal root state and posterior probability estimates resulting from different discrete Bayesian phylogeographic analyses applied to different datasets.

Method	Subsampling						
	Complete	**RAND10A**	**RAND10B**	**RAND10C**	**RAND20A**	**RAND20B**	**RAND20C**
pol							
Symmetric-BSSVS	FLP (1.00)	SPL (0.99)	CTB (0.99)	RJN (0.99)	POA (0.99)	RIG (0.99)	RIG (0.99)
Symmetric	FLP (0.99)	CRI (0.99)	FLP (0.97)	CRI (0.97)	FLP (0.99)	FLP (0.98)	ITA (0.99)
Asymmetric-BSSVS	FLP (1.00)	CRI (1.00)	FLP (0.99)	CTB (0.99)	ITA (1.00)	FLP (0.99)	POA (0.99)
Asymmetric	FLP (0.99)	RJN (0.99)	FLP (0.99)	CTB (0.99)	ITA (1.00)	FLP (0.99)	FLP (0.99)
env							
Symmetric-BSSVS	FLP (0.97)	FLP (0.99)	POA (0.99)	POA (0.99)	FLP (0.99)	FLP (0.99)	CXS (0.99)
Symmetric	FLP (0.97)	FLP (0.99)	POA (0.99)	FLP (0.96)	FLP (0.99)	FLP (0.99)	CRI (0.99)
Asymmetric-BSSVS	FLP (0.99)	CRI (0.99)	POA (0.99)	LAJ (0.99)	CRI (1.00)	POA (0.99)	FLP (1.00)
Asymmetric	FLP (0.99)	BLU (0.99)	FLP (0.99)	CRI (0.69)	FLP (0.99)	CRA (0.99)	FLP (1.00)

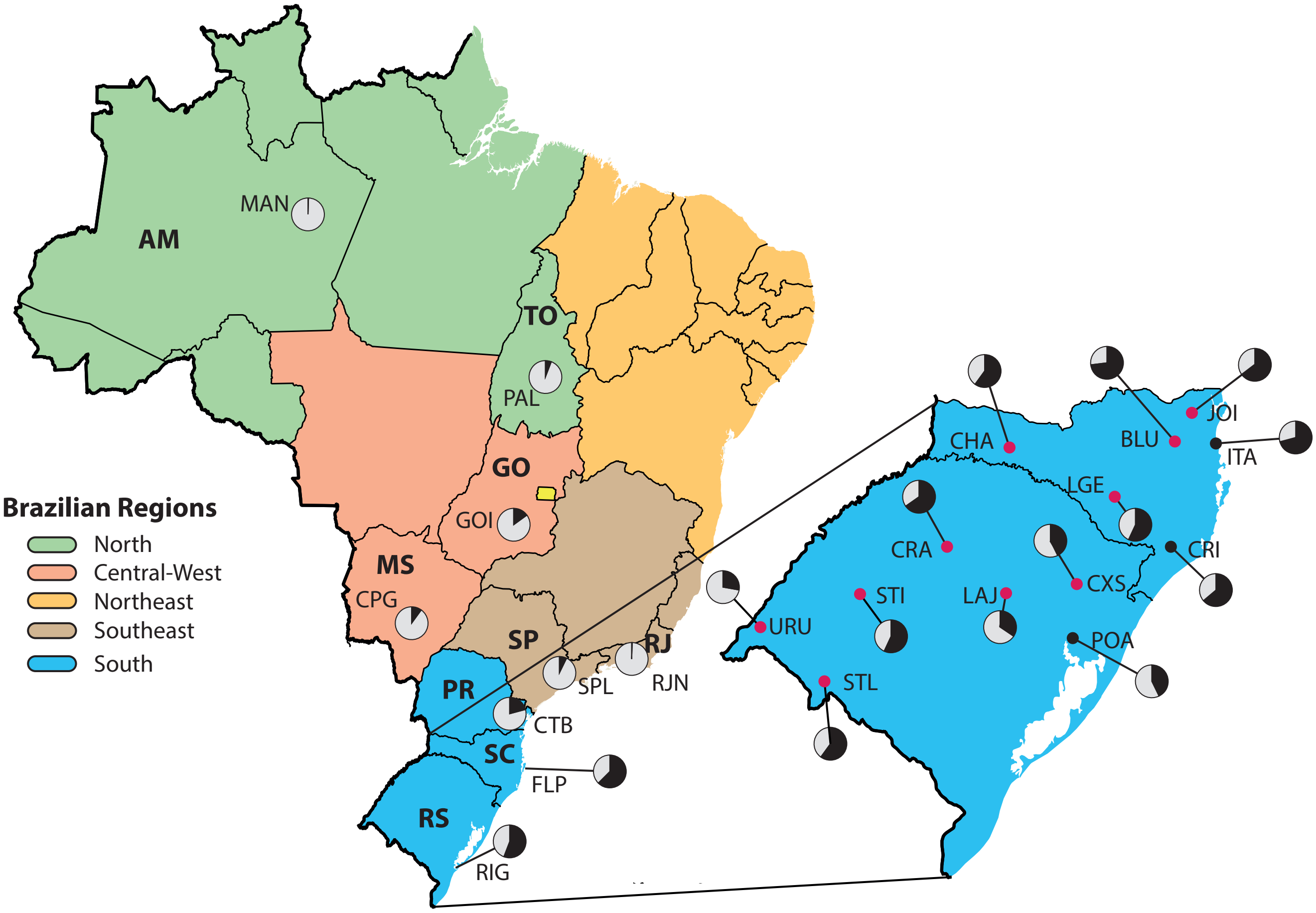
Acronyms for cities: BLU: Blumenau; CRI: Criciúma; CTB: Curitiba; CXS: Caxias do Sul; FLP: Florianópolis; ITA: Itajaí; LAJ: Lajeado; POA: Porto Alegre; RIG: Rio Grande; RJN: Rio de Janeiro.

Table 2. Bayes factor support for an explanatory role in the HIV-1C_BR diffusion process for all tested predictors in all datasets

Predictor				Dataset			
	Complete	RAND10A	RAND10B	RAND10C	RAND20A	RAND20B	RAND20C
pol							
Geographical Distance	0.1	0.0	0.0	0.1	0.0	0.0	0.0
Origin Sample Size	**Inf**	0.5	0.4	0.4	0.4	0.4	0.4
Destination Sample Size	**Inf**	**6583.3**	**5758.4**	**1674.5**	**Inf**	**Inf**	**Inf**
Origin HIV pop. size	0.6	1.4	1.1	1.6	1.2	1.1	1.2
Dest. HIV pop. size	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Origin HIV prevalence	0.2	**6.1**	**10.8**	**16.1**	**17.3**	**13.1**	**7.9**
Dest. HIV prevalence	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Origin HIV-1C pop. size	0.3	**14.4**	**22.4**	**9.6**	**18.1**	**23.4**	**38.8**
Dest. HIV-1C pop. size	0.0	0.0	0.1	0.1	0.0	0.0	0.0
Origin HIV-1C prevalence	0.3	**7.2**	1.3	**4.1**	1.1	1.4	1.0
Dest. HIV-1C prevalence	0.0	0.0	0.0	0.0	0.0	0.0	0.0
env							
Geographical Distance	0.0	0.1	0.1	0.1	0.0	0.0	0.0
Origin Sample Size	**Inf**	0.3	0.4	0.9	0.3	0.3	0.4
Destination Sample Size	**Inf**	**20.6**	**32.5**	**29.4**	**Inf**	**Inf**	**Inf**
Origin HIV pop. size	0.3	0.8	1.1	2.6	0.8	0.9	0.9
Dest. HIV pop. size	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Origin HIV prevalence	0.3	**19.6**	**18.9**	**15.3**	**22.3**	**26.0**	**23.4**
Dest. HIV prevalence	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Origin HIV-1C pop. size	0.3	**13.8**	**14.0**	**15.5**	**13.0**	**11.3**	**12.9**
Dest. HIV-1C pop. size	0.0	0.1	0.1	0.0	0.0	0.0	0.0

Origin HIV-1C prevalence	0.4	0.4	0.6	1.0	0.7	0.6	0.5
Dest. HIV-1C prevalence	0.0	0.6	0.6	0.6	0.0	0.0	0.0

Epidemiological predictors included in all Rand10 and Rand20 datasets are in bold, as well as BF ≥3; Dest. : destination.


Brazilian Regions
North
Central-West
Northeast
Southeast
South
AM
MAN
TO
PAL
GO
GOI
MS
CPG
SP
SPL
RJ
RJN
PR
CTB
SC
FLP
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CRI
URU
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LAJ
POA
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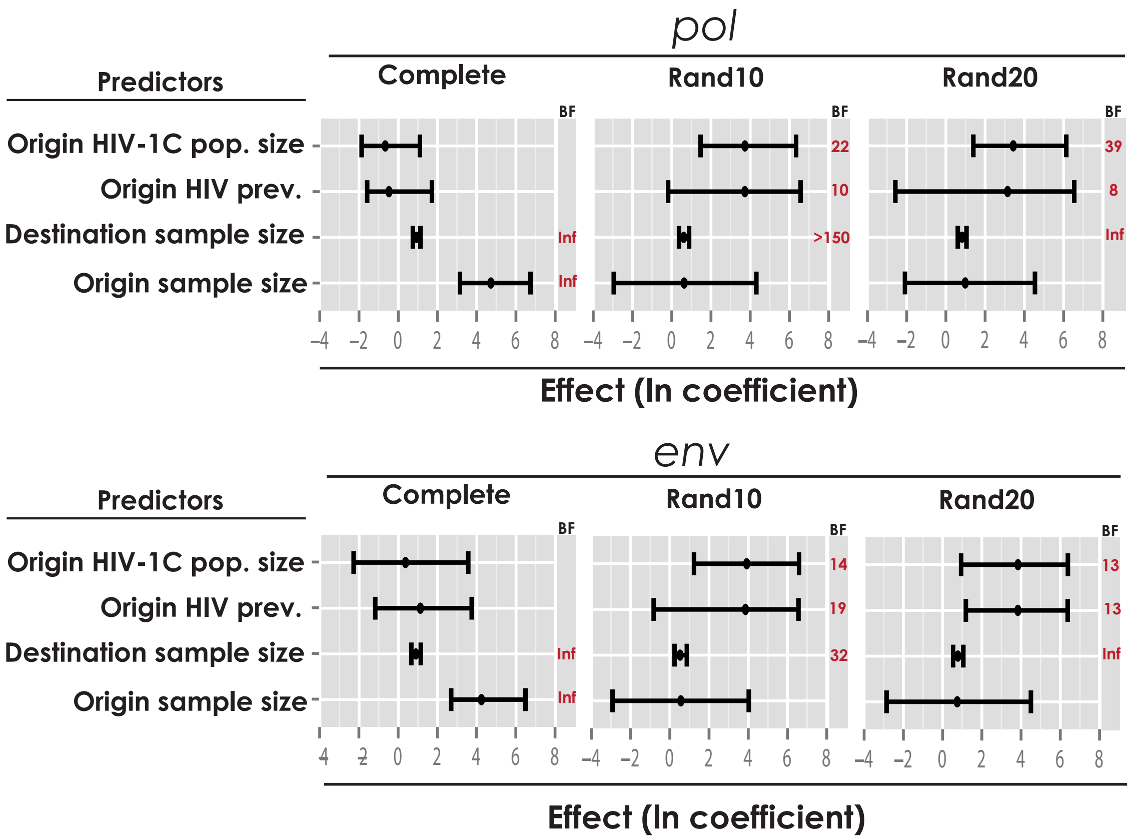
pol
Predictors
Complete
Rand10
Rand20
BF
Origin HIV-1C pop. size
Origin HIV prev.
Destination sample size
Origin sample size
22
10
>150
39
8
Inf
Inf
Inf
−4 −2 0 2 4 6 8
Effect (ln coefficient)
env
Predictors
Complete
Rand10
Rand20
BF
Origin HIV-1C pop. size
Origin HIV prev.
Destination sample size
Origin sample size
14
19
32
13
13
Inf
Inf
Inf
−4 −2 0 2 4 6 8
Effect (ln coefficient)