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Rapid Communication

Explosive HIV-1 subtype B' epidemics in Asia driven by geographic and risk group founder events

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ABSTRACT

We explored the timescale, spatial spread, and risk group population structure of HIV-1 subtype B', the cause of explosive blood-borne HIV-1 epidemics among injecting drug users (IDUs) and former plasma donors (FPDs) in Asia. Sequences from FPDs in China formed a distinct monophyletic cluster within subtype B'. Further analysis revealed that subtype B' was founded by a single lineage of pandemic subtype B around 1985. Subsequently, the FPD cluster appears to have derived from a single subtype B' lineage around 1991, corroborating the hypothesis that FPD outbreaks stemmed from the preceding epidemic among IDUs in Southeast Asia, most likely from the Golden-Triangle region.

Introduction

HIV-1 subtype B' (Thailand variant of subtype B; also referred to as Thai-B or B$_B$) (Kalish et al., 1995; Ou et al., 1993; Weniger et al., 1994) is a unique regional variant of subtype B that has caused explosive epidemics in Asia via the routes of blood-borne transmission, namely networks of injecting drug users (IDUs) (Ou et al., 1993) and unhygienic plasma collection (Mastro and Yip, 2006). The strain was originally identified among IDUs in Bangkok, Thailand (Ou et al., 1993) and dominated the early phase of the HIV epidemic in Bangkok that began in December 1987 (Kalish et al., 1995; Ou et al., 1993). However, as the epidemic matured, the proportion of CRF01_AE infections in Bangkok increased relative to B', to the extent that in 1995–1998 about 80% of new IDU infections in the city were caused by CRF01_AE (Subbarao et al., 2000). In contrast, subtype B' is almost the only strain found among IDUs in Yangon, the capital city of Myanmar, and even predominates among heterosexuals in Myanmar, accounting for more than 30% of infections (Kusagawa et al., 1998; Motomura et al., 2003).

It has also reported that subtype B' is a single founder strain responsible for a series of HIV-1 outbreaks among former plasma donors (FPDs) in Central China (Deng et al., 2008; Zhang et al., 2004). An estimated 250,000 people, mostly rural peasants, were infected through unhygienic plasma collection from the early 1990s (from ~1992) until 1996, when the practice was banned (Mastro and Yip, 2006). The most heavily affected provinces were Henan, Anhui, Hubei and Shandong in Central China (Mastro and Yip, 2006).

In addition to being an epidemiologically important strain by itself, subtype B' is also a constituent of up to six different circulating recombinant forms (CRFs) in Asia, most notably CRF07_BC (Su et al., 2000) and CRF08_BC (Piyasirisilp et al., 2000) in China, which are descendants of subtypes B' and C. Four CRFs comprised of CRF01_AE and subtype B' have been reported to date: CRF15_01B (Tovanabutra et al., 2003) and CRF34_01B (Tovanabutra et al., 2007) from Thailand: CRF33_01B (Tee et al., 2006) and CRF48_01B (Li et al., in press) from Malaysia (http://www.hiv.lanl.gov/content/sequence/HIV/CRFs/).

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Furthermore, in addition to these CRFs, various unique recombinant forms (URFs) that harbor subtype B' genetic material have been found in Asian countries, including Thailand (Kijak et al., 2007), Myanmar (Takebe et al., 2003), Malaysia (Tee et al., 2006; Wang et al., 2007) and the Yunnan province of China (Qiu et al., 2005; Yang et al., 2002).

Recently developed phylogenetic and molecular clock methods can be used to reconstruct the epidemic history of HIV and to estimate the time of the common ancestors of specific strains (Drummond and Rambaut, 2007). Such evolutionary analyses help to enhance our understanding of the genesis and development of global and regional HIV-1 epidemics.

A previous study by Deng et al. estimated that the common ancestor of subtype B' existed around 1985 (Deng et al., 2008). Historical accounts suggest that subtype B' outbreaks began among IDUs in Thailand and neighboring regions in the late 1980s (Weniger et al., 1994), prior to the outbreaks among FPDs in China in early-mid 1990s (Mastro and Yip, 2006). In this study, we re-examined the space–time process of the overland dissemination of HIV-1 subtype B' – a strain that is uniquely associated with blood-borne transmission routes in Asia.

Results

To carry out the analysis, we selected a 1.6-kb *gag-pol* region (HXB2: 1789–3421) of subtype B' because this region maximizes the length of the nucleotide sequences, whilst simultaneously resulting in a sufficient number of sequences from various countries and risk populations from the database. A total of 92 of all available subtype B' sequences, sampled between 1994 and 2006, were obtained from the Los Alamos HIV Sequence Database (www.hiv.lanl.gov). Sampling locations were Thailand ($n=6$), Myanmar ($n=1$), Yunnan (south-western China) ($n=10$), Henan (Central China) ($n=4$), Hubei (Central China) ($n=45$), and Liaoning (Northeastern China) ($n=26$).

Using the molecular clock approach implemented in BEAST v1.4 (Drummond and Rambaut, 2007), we estimated the timescale of subtype B' evolution from the known sequence sampling dates, which ranged from 1983 to 2005. Estimations were obtained using a Bayesian Markov chain Monte Carlo (MCMC) method under various nucleotide substitution and evolutionary models (Table 1). The estimated evolutionary rates were 3.1 (2.5–3.8) $\times 10^{-3}$ and 3.4 (2.7–4.0) $\times 10^{-3}$ substitutions/site/year for GTR + Γ4 and HKY + Γ4 models with a constant size coalescent model (see Materials and Methods) and 3.0 (2.5-3.5) $\times 10^{-3}$ and 3.1 (2.6–3.6) $\times 10^{-3}$ substitutions/site/year for GTR + Γ4 and HKY + Γ4 models with a skyline coalescent model (Table 1).

As shown in a maximum clade credibility (MCC) tree in Fig. 1, HIV-1 subtype B' circulating among FPDs (designated B'FPD) in Henan, Hubei and Liaoning formed a monophyletic cluster within subtype B'. In other words, subtype B' showed paraphyletic relationship with respect to B'FPD, indicating that B'FPD is a descendant lineage of subtype B'.

The Bayesian relaxed molecular clock analysis estimated the date of the common ancestor of subtype B' to be 1984.5 (95% credible region, CR: 1980.9–1987.7) (Fig. 1). This is in good agreement with Deng et al.'s previous estimate (Deng et al., 2008). In contrast, the likely year of origin of the B'FPD clade was estimated at 1991.2 (95% CR: 1989.1–1993.5) (Fig. 1, Table 1). We date the tMRCA of pandemic subtype B (B'PAN) to 1966.0 (95% CR: 1957.9–1973.1), consistent with the results of Gilbert et al. (2007). The evolutionary and statistical assumptions have no significant effect on the estimated dates (Table 1).

Discussion

In this study, we used newly developed analysis tools to investigate the temporal and spatial dynamics of HIV-1 subtype B' transmission, a regional variant of HIV-1 subtype B that caused explosive epidemics among IDUs in southeast Asia and FPDs in Central China.

As shown in Fig. 1, HIV-1 strains circulating among FPDs (B'FPD) formed a monophyletic cluster within subtype B'. As subtype B' is paraphyletic with respect to B'FPD, B'FPD is likely a descendant lineage of subtype B'. The Bayesian molecular clock analysis estimated the date of the common ancestor of subtype B' to be 1984.5 (95% CR: 1980.9–1987.7). In contrast, the estimated date of origin of the B'FPD clade was more recent, at 1991.2 (95% CR: 1989.1–1993.5) (Fig. 1, Table 1). Taken together, these results indicate that the epidemic among FPDs in China was triggered in early 1990s by a single lineage of subtype B' emerging from the IDU risk population.

In the present analysis, we chose a 1.6-kb *gag-pol* region (HXB2: 1789–3421) in order to maximize the phylogenetic resolution. In practice, this is the only region for which we can obtain a sufficient number of nucleotide sequences from various countries and risk populations (i.e., IDUs and FPDs) whilst concurrently maximizing the length of the nucleotide sequences available. In addition to this 1.6-kb *gag-pol* region, we also investigated 325 sequences representing a 351 nt region of the *env* gene (HXB2: 7050–7400) as well as 488 sequences representing a 485 nt region of *gag* p17 (HXB2: 790–1274). Analyses of these shorter regions suggest a general trend distinguishing B'FPD from subtype B' but did not contain significant statistical support for this separation. Deng et al.'s analysis (Deng et al., 2008) used comparatively short nucleotide sequences (483 nt of *gag* and 216 nt of *env*), which likely explains why we identified a distinct B'FPD subcluster in our study using 1.6-kb long sequences.

In the *gag-pol* phylogeny, the B' sequences that are most closely related to the B'FPD cluster are the isolates from western Yunnan province (Dehong district) (Fig. 1). We have previously shown that subtype B' is found exclusively in the western part (Dehong district) of Yunnan province (Fig. 2B), near the border with Myanmar, where the first HIV-1 epidemic among IDUs in China began in 1989 (Yang et al., 2002). It is thus tempting to speculate that a founder strain of the B'FPD cluster originated from one of the B' lineages present in western Yunnan and subsequently transferred to the FPD risk group in

Table 1
Estimated substitution rates and dates origin for HIV-1 subtype B/B' lineages.

Genetic region	Model[a]		Rate of evolution[b]	Coefficient of variation	Date of tMRCA year[c]		
					B (Pandemic)	B' (IDU)	B' (FPD)
gag-pol subtype B' (HXB2: 1789-3421)	GTR + Γ4	Constant	3.1 (2.5, 3.8)	0.5 (0.3, 0.6)	1965.0 (1953.2, 1974.3)	1984.1 (1980.7, 1987.8)	1991.1 (1988.6, 1993.4)
	HKY + Γ4	Constant	3.4 (2.7, 4.0)	0.5 (0.3, 0.6)	1965.8 (1955.8, 1974.1)	1984.0 (1980.4, 1987.7)	1991.2 (1988.8, 1993.4)
	GTR + Γ4	Skyline	3.0 (2.5, 3.5)	0.3 (0.3, 0.4)	1970.7 (1964.5, 1976.3)	1985.0 (1981.7, 1988.5)	1991.5 (1989.5, 1993.5)
	HKY + Γ4	Skyline	3.1 (2.6, 3.6)	0.3 (0.3, 0.4)	1970.7 (1965.0, 1975.8)	1984.7 (1981.4, 1988.1)	1991.6 (1989.5, 1993.5)
(Mean)					1968.1 (1959.6, 1975.1)	1984.5 (1981.1, 1988.0)	1991.4 (1989.1, 1993.5)

[a] Based on BEAST analysis under a relaxed molecular clock with a GTR + Γ4 or HKY + Γ4 substitution model and a constant size coalescent or skyline model.
[b] Estimates of the mean evolutionary rate (μ: $\times 10^{-3}$ nucleotide substitutions/site/year) for subtype B/B'.
[c] Mean time of the most common ancestor (tMRCA: year) for the subtype B/B' dataset [95% highest posterior density (HPD) in parentheses]. B (pandemic) = pandemic subtype B; B' (IDU) = subtype B' strains that is responsible for HIV-1 outbreaks among injecting drug users (IDUs) in Southeast Asia; B' (FPD) = subtype B' strains that is responsible for HIV-1 outbreaks among former plasma donors (FPDs) in Central China.

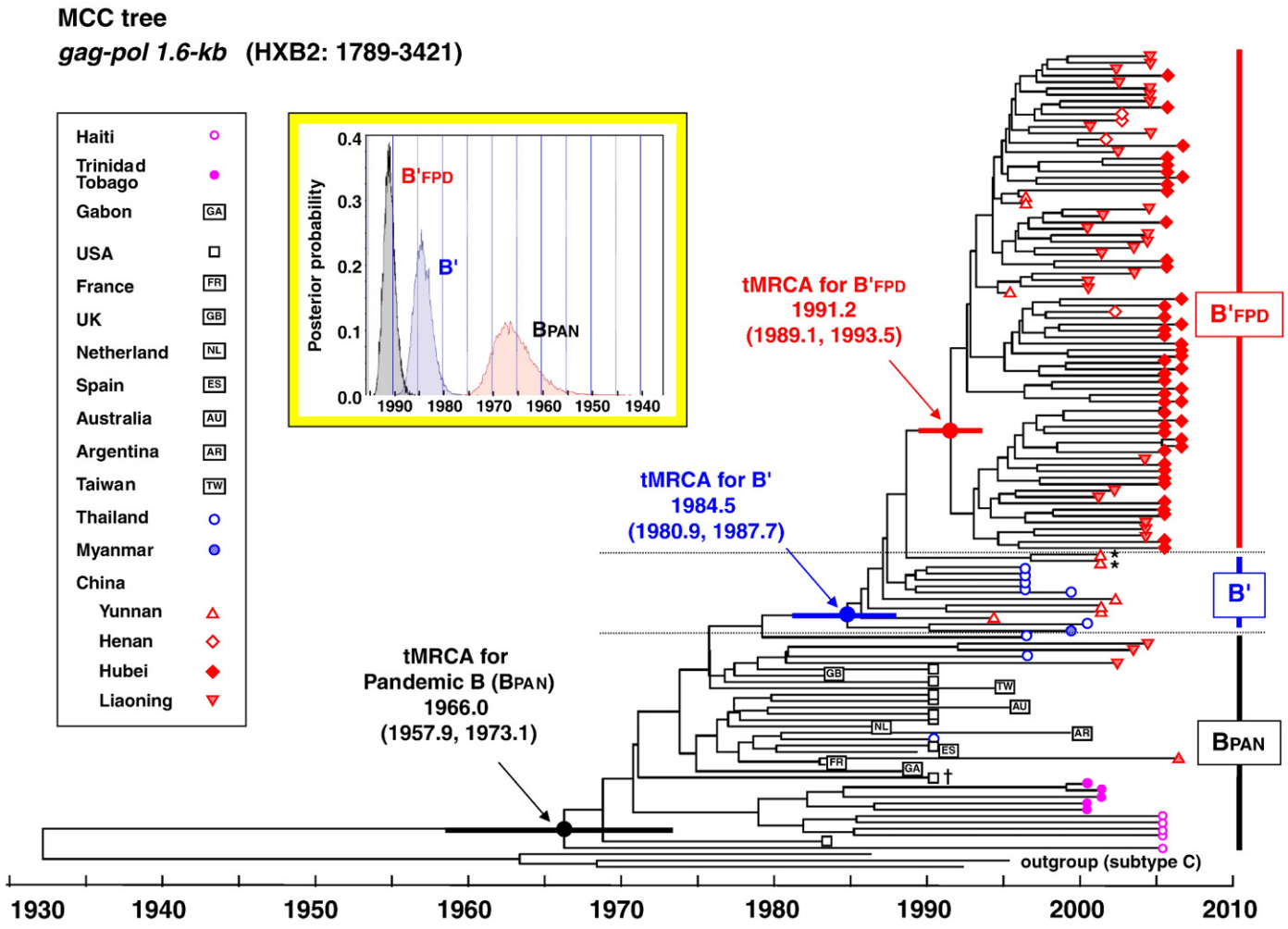

Fig. 1. Evolutionary characteristics of HIV-1 subtype B'. Maximum clade credibility (MCC) tree of 1.6-kb *gag*-RT sequences obtained by Bayesian MCMC analysis is shown (see texts for details). HIV-1 subtype C sequences (86ETH2220, 95IN21068 and 92BR025d) were used as outgroups. The tips of the tree correspond to the year of sampling, and the branch lengths reflect the mean of the posterior probability density. The 95% highest probability density (HPD) for each subtype B/B' cluster is indicated as a horizontal bar at the corresponding node. Inset, distribution of the posterior probability for the respective subtype B/B' lineage. The symbols for the geographic origins of the respective subtype B/B' strains are listed in the left-hand panel. The tMRCA means and 95% HPDs for the key nodes were as follows: pandemic subtype B ancestor = 1966 (1958-1973); subtype B' ancestor = 1985 (1981-1988); B'FPD ancestor = 1991 (1989-1994). The asterisk indicates two subtype B' isolates from western Yunnan (Dehong district). The dagger indicates one of the earliest subtype B isolate (RF) from a Haitian immigrant living in the United States. (Gilbert et al., 2007).

Central China. This process may be related to drug trafficking between the Golden triangle area and Central China (Fig. 2B).

Fig. 2 summarizes the epidemic history and plausible migration pathway of subtype B/B' lineages in Asia. As reported by Gilbert et al., subtype B moved from Africa to Haiti around ~1966 (1962–1970) and began to disperse around ~1969 (1966–1972), to the United States and elsewhere around the world (Gilbert et al., 2007). In Asia, subtype B' emerged from a pandemic subtype B lineage around ~1985, triggering an explosive epidemic among IDUs in Thailand and neighboring countries (including Myanmar, Western Yunnan, Malaysia and eastern India) (Weniger et al., 1994). Most recently, a specific variant of B' (B'FPD) emerged in ~1991 which triggered outbreaks among FPDs in Central China (Fig. 2).

Our study suggests that subtype B epidemics in Asia arose by the sequential introduction of founder strains into new locations and risk groups (Fig. 2B). There appear to be surprisingly few "successful" migration events, compared to the number of times that we might expect the virus to move from one place to another and from one risk population to another risk population. A similar phenomenon has been also observed in the global migration of CRF01_AE (Liao et al., 2009).

This remarkable epidemic success of subtype B' and B'FPD appears to reflect ecological/epidemiological factors rather than viral genetic factors (i.e., differences in transmission fitness), although we cannot formally rule out the possibility of selection at present. We do not observe any appreciable differences between HIV-1 subtype B, B' and B'FPD in their virological properties during growth in cell culture. Social factors, including pre-existing IDU networks and unhygienic plasma collection fueled the explosive spread of viruses in the regions, resulting in such profound founding effects. The analysis we reported here provides insights for in-depth understanding the origin and genesis of blood-borne HIV-1 epidemic in this particular region in Asia.

Materials and methods

Divergence time estimation

Investigation of the evolutionary history of the HIV-1 subtype B' strains was carried out using BEAST v1.4 (Drummond and Rambaut, 2007), in order to estimate the tMRCA of each phylogenetic cluster (Drummond et al., 2002; Pybus et al., 2003). The timescale of subtype B/B' evolution was estimated using a relaxed molecular clock model from the known sampling times of the B/B' sequences (Table 1 and Fig. 1). Relaxed-clock models have previously been shown to be more reliable in estimating viral phylogenies and divergence dates than

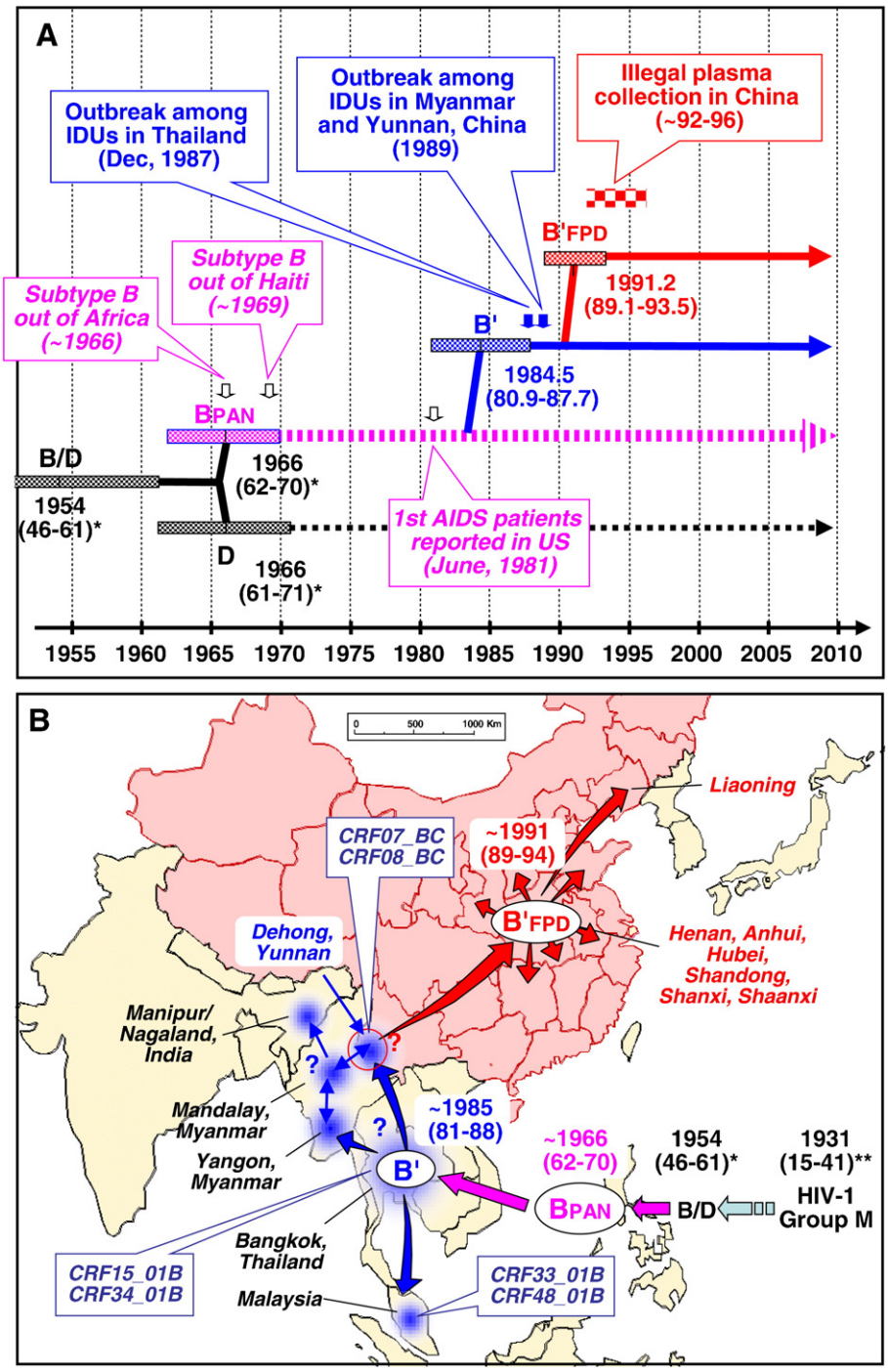

Fig. 2. Schematic representation of space–time dynamics of expansion of HIV-1 subtype B/B' lineages in Asia and the world. (A) Epidemic history of subtype B/B' lineages. The expected times of origin of the respective clusters are indicated with the historical landmarks relevant to the study. (B) Plausible pathway and timing of dispersal of subtype B' in Asia. B'PAN, Pandemic subtype B; B', subtype B' triggering IDU epidemic in Southeast Asia; B'FPD, subtype B' variant distributed among former plasma donors (FPDs) in Central China. The plausible sites of the emergence of six CRFs known to date in which subtype B' is a constituent are shown in the map. The estimated tMRCAs (asterisked) for HIV-1 subtype B/D common ancestor (*) (Gilbert et al., 2007) and HIV-1 group M (**) (Korber et al., 2000) are indicated according to the respective references.

"strict clock" and "non-clock" methods (Drummond et al., 2006; Lemey et al., 2006). Dates were estimated using Bayesian MCMC inference under a variety of coalescent and substitution models. Both the general time-reversal (GTR) (Rodriguez et al., 1990) and Hasegawa–Kishino–Yano (HKY) (Hasegawa et al., 1985) nucleotide substitution models, plus a gamma-distribution model of among site rate heterogeneity (with four rate categories) (Yang, 1994), were investigated. The relaxed clock model used was the uncorrelated lognormal model (Drummond et al., 2006). Two coalescent models of population size were applied: the constant size model and the Bayesian skyline plot model (Table 1). The analysis was computed for 20 million states sampled every 10000 states. The MCMC output was tested for convergence and effective sample size using Tracer v1.4 (available from http://beast.bio.ed.ac.uk).

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