

## Acknowledgements

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The study was approved by the research ethics committees of Université Laval, Québec, Canada and of the Ministry of Health of Côte d'Ivoire. Informed written consent was obtained from all participants.

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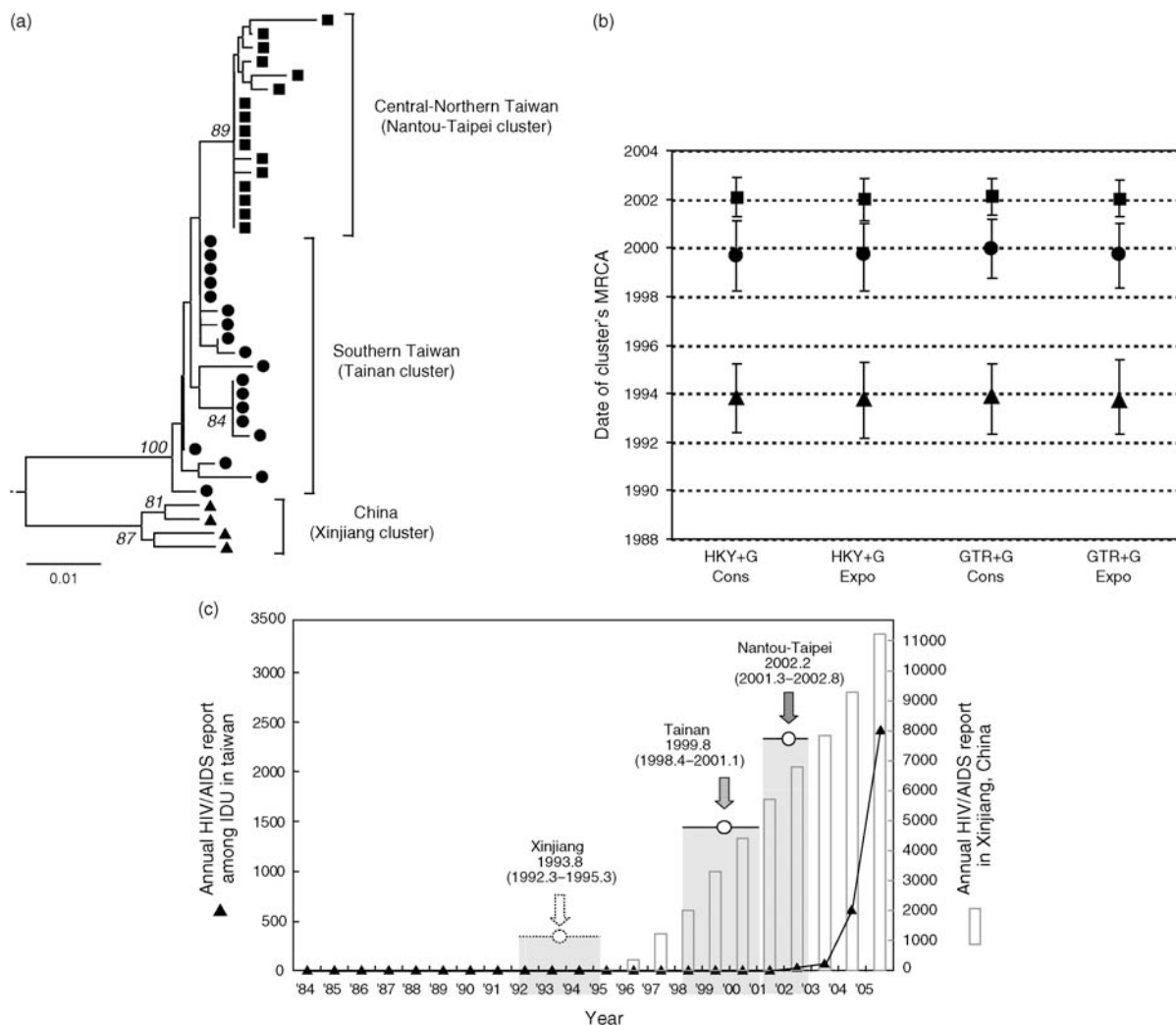
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## Chronology of the HIV-1 CRF07\_BC expansion in East Asia

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**The HIV-1 epidemic among injecting drug users (IDU) in Taiwan is caused primarily by CRF07\_BC infections. Evolutionary analyses, which utilize outgroup reference strains from northwestern China (Xinjiang), reveal that CRF07\_BC was introduced into southern Taiwan in 1998–2001 and spread to central–northern Taiwan in 2001–2003, causing the largest HIV/AIDS epidemic in Taiwan. The separate introduction of CRF07\_BC into Xinjiang occurred in 1992–1995. This study illustrates the temporal dynamics of CRF07\_BC spread among IDU across east Asia.**

The HIV-1 circulating recombinant form 07\_BC (CRF07\_BC) is a recombinant comprised of HIV-1 subtype B' (Thailand genotype of subtype B) and subtype C. This strain accounts for most infections among injecting drug users (IDU) in northwestern China (Xinjiang province), where an outbreak was detected in 1996 [1], although the most plausible origin of CRF07\_BC as a whole is probably Yunnan province [2,3]. Outside mainland China, CRF07\_BC is associated with a dramatic increase in HIV/AIDS cases in Taiwan; it was detected among IDU in prisons in southern Taiwan (Tainan) in 2002 and later detected in 2003–2004 in central (Nantou) and northern (Taipei) Taiwan [4]. By



**Fig. 1. Phylogenetic and evolutionary analysis of HIV-1 sequences from injection drug users in mainland China and Taiwan.** (a) Estimated phylogeny for the *env* gene of HIV-1 CRF07\_BC (HXB2 7077–7665 nt). For visual clarity, sequences are labeled with symbols according to location of isolation. (b) Estimated dates of the most recent common ancestors (MRCA) of CRF07\_BC sequences from China (Xinjiang), southern Taiwan (Tainan), and central–northern Taiwan (Nantou–Taipei). Vertical lines denote the 95% highest posterior density credible intervals. Dates were estimated under various nucleotide substitution and evolutionary models: HKY, Hasegawa–Kishino–Yano model; GTR, general time reversible model; G, gamma distributed among-site rate heterogeneity; Cons, constant population size; Expo, exponential population growth. ■ Central–northern (Nantou–Taipei) cluster; ● southern Taiwan (Tainan) cluster; ▲ China (Xinjiang) cluster; (c) Change in annual HIV/AIDS cases in Xinjiang, China (□) and Taiwan (▲) between 1984 and 2005. The time to the MRCA of CRF07\_BC clusters detected in the indicated locales are depicted with 95% highest posterior density credible intervals (in parentheses).

the end of September 2007, 15 183 HIV cases have been reported in Taiwan since 1984 and the majority of infections occurred relatively recently among IDU [5]. CRF07\_BC accounts for 98% of the infections among IDU in Taiwan [4].

To estimate the timescale of CRF07\_BC spread in mainland China (Xinjiang) and in Taiwan, we performed phylogenetic and Bayesian coalescent analyses on *env* sequences obtained from GenBank. The CRF07\_BC *env* sequences used in this study are from Xinjiang province, China ( $n=4$ ; sampled in 1997–1998), and from three cities in Taiwan; in particular,

Tainan (southern;  $n=19$ ), Nantou (central;  $n=8$ ) and Taipei (northern;  $n=8$ ), all sampled in 2004 (accession numbers EF078077–EF078079, EF078082–EF078105 and EF078107–EF078114). As shown in Fig. 1a, the CRF07\_BC sequences group into three distinct phylogenetic clusters, denoted Xinjiang (China), Tainan (southern Taiwan) and Nantou–Taipei (central and northern Taiwan). Using the molecular clock approach implemented in BEAST v1.4 [6], we estimated the rate of evolution of the hypervariable region-stripped *env* gene (HXB2 7077–7665 nt) from an independent dataset of 41 HIV-1 subtype C strains with known sampling dates that ranged from 1989 to 2005. The

evolutionary rate estimate obtained ( $4.7\text{--}5.0 \times 10^{-3}$  substitutions per site per year) was then incorporated as a prior probability distribution in the analysis of the CRF07\_BC sequences [7]. A Bayesian Markov chain Monte Carlo method was used to estimate the dates of the most recent common ancestors (MRCA) of CRF07\_BC in Xinjiang and Taiwan under various nucleotide substitution and evolutionary models. As illustrated in Fig. 1b, the likely year of origin of CRF07\_BC from Xinjiang, China, was August 1993 [95% credible region (CR) March 1992–March 1995]. MRCA of CRF07\_BC strains from southern and central-northern Taiwan were dated to August 1999 (95% CR April 1998–January 2001) and February 2002 (95% CR March 2001–August 2002), respectively. The evolutionary and statistical assumptions used have almost no effect on the estimated dates (Fig. 1b).

This study indicates that CRF07\_BC was introduced into the IDU population in Xinjiang in the early to mid 1990s (1992–1995). The strain also spread into IDU in southern Taiwan in the late 1990s (1998–2001), and subsequently disseminated northward to IDU in central–northern Taiwan in the early 2000s (2001–2003), resulting in the largest ever HIV/AIDS epidemic in Taiwan. HIV/AIDS surveillance detected a dramatic upsurge of HIV cases in Xinjiang in the mid-1990s (1995–1996), and in 2003–2004 in Taiwan (Fig. 1c), suggesting that CRF07\_BC may have been present among IDU for a year or two in each region before the epidemic spread and subsequent detection of the infection. It is most likely that both the Xinjiang and Taiwan outbreaks trace their origins back to Yunnan province, thought to be the geographical origin of CRF07\_BC [2,3]. We note, however, that MRCA dates can also be more recent than the date of outbreak discovery, either because extant virus diversity has been incompletely sampled, or because the founding lineages of the outbreak have since gone extinct [8,9]. These results illustrate the history of the regional and international spread of HIV-1 CRF07\_BC in east Asia.

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