GENOME WATCH

An elephantine viral problem

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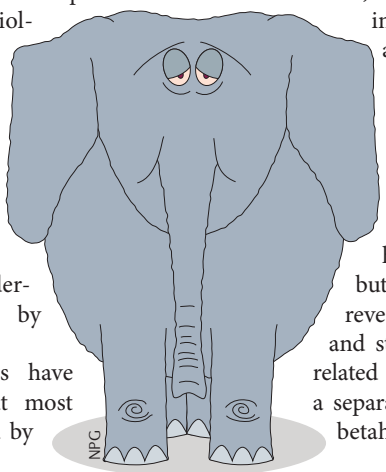
This month's Genome Watch highlights how deep sequencing was used to generate the first full genomes of herpesviruses associated with a fatal disease in elephants.

Elephant endotheliotropic herpesviruses (EEHVs) infect elephant endothelial cells and can cause a fatal haemorrhagic disease. The disease affects mainly young Asian elephants and has a mortality rate of more than 80%. Asian elephants are an endangered species, and wild populations are decreasing as natural habitats are lost. At the same time, a number of EEHV infections have been reported in captive animals. Understanding EEHV transmission and pathogenesis might help to protect these rare animals.

In 1999, Richman *et al.*[1] suggested that the disease emerges by cross-species transmission from African to Asian elephants when the two species are housed in close proximity. Identical viral sequences were isolated from otherwise healthy African elephants with external skin lesions and Asian elephants with haemorrhagic disease. The authors hypothesized that EEHVs are latent and benign in African elephants, but lethal when they enter the new host species. However, the epidemiology of the disease is more complicated; a number of healthy Asian carriers and a few fatal infections of African elephants were also described. Thus, more viral (and host) genetic information is needed to determine mechanisms underlying the disease caused by EEHVs.



Eight EEHV genotypes have been reported so far, but most lethal infections are caused by EEHV1A and EEHV1B. EEHVs belong to the Proboscivirus genus, which is grouped with other herpesviruses such as cytomegaloviruses and roseoloviruses in the subfamily Betaherpesvirinae. Two new studies[2,3] now report the first complete genome sequences for EEHVs and shed new light on the relationships of these viruses with other herpesviruses. Wilkie *et al.*[2] used deep sequencing to generate complete EEHV genomes from two Asian elephants, Raman and Emelia, that died in British zoos. EEHVs do not grow in tissue culture, so DNA was isolated and sequenced directly from heart and tongue tissue. Data analysis was challenging owing to the lack of both an Asian elephant and an EEHV reference genome. However, *de novo* assembly and joining of contigs using several programmes was combined with PCR and Sanger sequencing of specific regions such as repeats, allowing the authors to generate the complete sequences of EEHV1A str. Raman and EEHV1B str. Emelia. Ling *et al.*[3] used a similar approach to determine an EEHV1A genome sequence directly from liver tissue of the Asian elephant Kimba, which died in the USA.

The genomes are 180,421 bp (EEHV1 str. Raman), 180,358 bp (EEHV1 str. Emelia) and 177,136 bp (EEHV1 str. Kimba) in length, with 116, 115 and 115 predicted genes, respectively, and consist of large unique regions flanked by direct terminal repeats. The EEHV genome structure is similar to that of human roseoloviruses, but phylogenetic analysis revealed that strain Raman and strain Emelia are closely related to each other and form a separate clade outlying other betaherpesviruses[2]. Ling *et al.* found that 60 of the predicted genes in strain Kimba are not present in any known herpesvirus, and proposed that EEHVs be classified as a new subfamily, Deltaherpesvirinae. Wilkie *et al.* also noted that almost half the EEHV genes lack orthologues in other betaherpesviruses. Understanding the function of these novel genes could provide important clues for understanding the transmission and pathogenesis of these herpesviruses, and in particular their endothelial cell tropism.

These first two studies will allow faster and more cost-effective future studies of full EEHV genomes. Only a tiny proportion (0.169% and 0.038%, respectively) of the total reads generated by Wilkie *et al.* were derived from the virus. For human herpesviruses, efficient deep sequencing of large numbers of genomes can be achieved with a target capture method that specifically enriches for viral genomes[4]. Now that certain full EEHV genomes are known, this hybridization-based capture method can be used to determine additional EEHV genomes, which will facilitate the development of better diagnostics and maybe even vaccines against this fatal disease threatening Asian elephants.

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Competing interests statement
The authors declare no competing financial interests.