Truck drops

One of the problems facing the introduction of novel crops is the concerns about their spread into the natural environment. A long-term study of oilseed rape (canola) growing on the verges of one of Britain's busiest motorways provides some encouragement that at least some crops find the going tough outside the field environment in which they are sown.

Michael Crawley at Imperial College London and Susan Brown at the Winfrith Technology Centre in Dorchester (published in the Proceedings of the Royal Society online) have studied populations of oilseed rape growing in more than 3,500 quadrants at the side of London's M25 motorway for ten years.

The researchers found oilseed rape showed a wide range of temporal dynamics over the study period (decreases, increases, cycles, extinction, re-colonization and stasis). The most frequently observed pattern, however, involved classic 'casual' dynamics with populations lasting for just 1 or 2 years before local extinction.

Part of their apparent persistence was due to spillage of seed from lorries taking oilseed rape to a processing plant. The crop was much less evident on the opposite carriageway leading away from the plant.

The work suggests some crop plants may find it much tougher than is apparent when they go wild.

Seeds of confusion: Loss of seed from lorries taking oilseed rape on the way to a processing factory near London masked the fact that feral populations of this species tend to be very short-lived. (Photograph: Associated Press.)

Correspondence

Tumor outbreaks in marine turtles are not due to recent herpesvirus mutations

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Marine turtle fibropapillomatosis is a devastating, transmissible disease characterized by multiple cutaneous and visceral fibrovascular tumors [1]. It has emerged with high prevalence since the 1980s and threatens the survival of several species of marine turtles. A herpesvirus, here called chelonid fibropapilloma-associated herpesvirus (C-FP-HV), is present in all naturally occurring tumors but not in unaffected turtles. It is also present in all tumors experimentally induced with cell-free tumor filtrates [2–5].

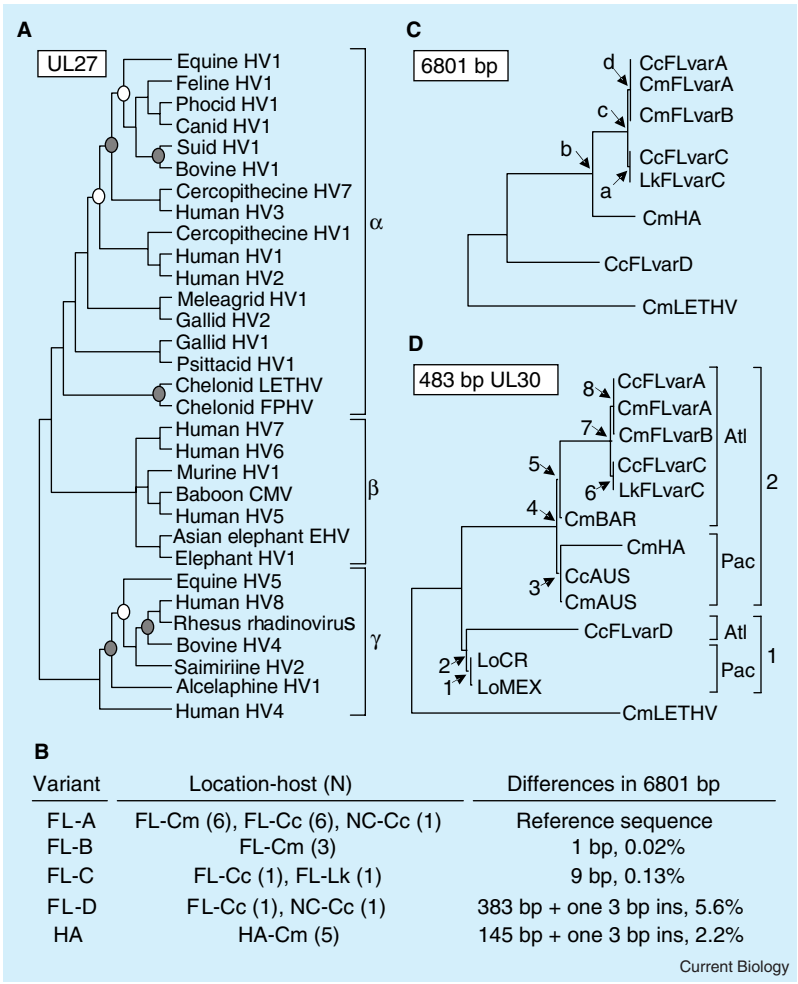
We assembled 43,843 bp of sequence of the C-FP-HV genome (GenBank AY644454), which encompasses 20 genes that are orthologous to cognate genes of other alpha-herpesviruses and organized in a similar fashion. In addition, the sequence included a novel 4 kb segment between UL15B and UL18 for which there is no equivalent in other herpesviruses (Supplemental Data). Phylogenetic analyses based on UL27, which has been widely used for herpesvirus phylogeny [6], show that C-FP-HV is related to other alpha-herpesviruses. It is most closely related to a non-oncogenic, marine turtle herpesvirus, C-LET-HV (Figure 1A). This UL27-based phylogeny agrees with that based on parts of UL29 and UL30 [3,7,8]. Alpha-herpesviruses are known to show extensive coevolution with their hosts [9–11]. The turtle virus lineage appears to have diverged before the separation of avian and mammalian alpha-herpesviruses (Figure 1A), suggesting that these herpesviruses have been unique

to turtles since they diverged from other vertebrates roughly 300 million years ago (mya) [12]. An alternative explanation is that an ancestral alpha-herpesvirus crossed host lineages sometime after they diverged.

To determine whether single viral variants could be linked to regional outbreaks, we compared C-FP-HV from fibropapilloma tumors in 25 turtles representing three species (green turtle, *Chelonia mydas*; loggerhead, *Caretta caretta*; and Kemp's ridley, *Lepidochelys kempii*). Five fragments encompassing 6801 bp of the viral genome could be amplified from tumors, but not normal tissue. However, unexpectedly, we detected five viral variants (Figure 1B), three of which, from Florida (A, B and C), are nearly identical. Surprisingly, variant D isolated from loggerhead turtles in Florida and North Carolina differs by 5.6% from the others, whereas the Hawaiian variant (HA) differs from the Florida variants A, B and C by only 2.2%. Thus, a variant (D) that differs considerably from variants A, B, and C was present in tumors from the same locales.

Interestingly, variant A was found in six green turtles and seven loggerheads, while variant C was detected in a loggerhead and a Kemp's ridley (Figure 1B). This shows that each variant crossed between sympatric species after all the nucleotide substitutions unique to variants A and C had occurred. Also, this is the first report of C-FP-HV in a Kemp's ridley, the most endangered turtle species.

Phylogenetic analyses were performed on the 6801 bp that defined the five variants to investigate their relationships (Figure 1C). This indicated that the Florida variant D forms a separate clade, which has diverged from the other C-FP-HVs before the Hawaiian variant separated from the Florida variants A, B and C. To explore C-FP-HV relationships further, we used 483 bp of UL30 sequence from five additional viral sequences available in GenBank. The resulting tree (Figure 1D) has the same topology as the one based on 6801 bp and reveals that variant D from Atlantic loggerheads is more closely related to C-FP-HV from Pacific olive ridleys than to other Atlantic, Caribbean or Hawaiian variants. Thus, our results strongly support a model in which a viral lineage, 'clade 1', leading to variant D and the olive ridley isolates, diverged from a 'clade 2', consisting of



B

Variant	Location-host (N)	Differences in 6801 bp
FL-A	FL-Cm (6), FL-Cc (6), NC-Cc (1)	Reference sequence
FL-B	FL-Cm (3)	1 bp, 0.02%
FL-C	FL-Cc (1), FL-Lk (1)	9 bp, 0.13%
FL-D	FL-Cc (1), NC-Cc (1)	383 bp + one 3 bp ins, 5.6%
HA	HA-Cm (5)	145 bp + one 3 bp ins, 2.2%

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Figure 1. C-FP-HV phylogeny.
(A) Phylogenetic analysis of herpesviruses (HV) from a wide taxonomic sample based on UL27 sequences. Alpha-, beta- and gamma-herpesvirus lineages are indicated. Nodes without ovals are supported at 100% bootstrap and jackknife proportions, and Bayes probabilities of 1.0. Filled ovals indicate proportions between 60% and 80% and probabilities between 0.6 and 0.8; open ovals indicate proportions <60% and probabilities <0.6. (B) Viral variants identified in this study based on 6801 bp of viral sequence (GenBank accession AY646888–AY646922). Five viral variants were identified from turtles from Florida (FL), Hawaii (HA), and North Carolina (NC). Three species of turtles were examined, green turtle (*Chelonia mydas*, Cm), loggerhead (*Caretta caretta*, Cc), and Kemp's ridley (*Lepidochelys kempii*, Lk). The number (N) of individual turtles for each location–species pair is indicated. Pairwise base pair differences were calculated relative to the reference sequence (FL-A), insertions (ins). Different 3 bp insertions were present in FL-D and HA. (C) Phylogenetic analysis of C-FP-HV variants based on 6801 bp from five amplicons (Supplemental data). Amplification of the two products involving the 4 kb insert required primers specific for either variant D or variant HA, in addition to a common primer pair for the closely related variants A, B, and C. When tumor samples were tested with all three pairs, only one yielded a product, thus indicating that the individual turtles in this study were each infected with only one variant. Bootstrap proportions, jackknife proportions and Bayes probabilities for nodes a to d were: a: 84, 85, 100; b: 86, 91, 100; c: 84, 85, 100; d: 84, 86, 100. (D) Phylogenetic analysis of C-FP-HV's based on 483 bp of UL30. Abbreviations are as in panel C plus olive ridley (*Lepidochelys oliviciae*, Lo), Australia (AUS), Barbados (BAR), Costa Rica (CR), Mexico (MEX). Clades 1 and 2 with Atlantic (Atl) and Pacific (Pac) sublineages within each are indicated. Bootstrap proportions, jackknife proportions and Bayes probabilities for nodes 1 to 8 were: 1: 78, 70, 73; 2: 68, 58, 53; 3: 97, 90, 89; 4: 95, 94, 95; 5: 92, 89, 97; 6: 51, 50, 53.

other C-FP-HV's (Figure 1D). Each clade contains Atlantic and Pacific variants.

Based on rates of nucleotide substitution estimated for human herpesviruses (1.4×10^{-8} to 3.5×10^{-8} per site per year) [9,13,14], we estimated that the 2.2% divergence between the Florida variants A, B and C and the Hawaiian variant occurred approximately 0.6–1.6 mya, whereas the 5.6% divergence between clades 1 and 2 occurred 1.6–4.0 mya. A plausible, alternative assumption holds that the rising of the Isthmus of Panama approximately 3.1–3.5 mya [15] prevented exchange of viruses between Atlantic/Caribbean and Pacific regions. Based on this, we hypothesize that the 2.2% difference between green turtle C-FP-HV's in Florida and Hawaii dates from 3.1–3.5 mya, and that variant D diverged from variants A, B, and C about 7.9–8.9 mya. This implies that the substitution rate of the turtle herpesvirus is two to five times slower than that of the human herpesviruses. Interestingly, turtle mitochondrial DNA mutation rates also appear to be slower [16]. Extrapolating from either approach, the interspecies transfers of variants A and C occurred anytime between a few thousand years ago and now.

The data and analyses presented here provide evidence that C-FP-HVs have been associated with their turtle hosts for millions of years. The existence of at least four viral lineages that have been separate for much of this time must be reconciled with the relatively recent emergence within the last few decades of epizootic fibropapillomatosis in multiple turtle species in multiple locations around the world [1]. Our data strongly argue that the current turtle fibropapillomatosis outbreaks are extremely unlikely to be due to recent virulence mutations in the C-FP-HV genome, as the likelihood of recent parallel mutations in four viral lineages is improbably small. Our findings also argue that the outbreaks are not due to worldwide spread of a single, emergent, pathogenic viral variant. Instead, they suggest that environmental or ecological factors underlie the current panzootic.

Supplemental data

Supplemental data are available at http://www.current-biology.com/cgi/content/full/14/17/R697/DC1/

References

1. Herbst, L.H. (1994). Fibropapillomatosis of marine turtles. Annu. Rev. Fish Dis. *4*, 389–425.
2. Lackovich, J.K., Brown, D.R., Homer, B.L., Garber, R.L., Mader, D.R., Moretti, R.H., Patterson, A.D., Herbst, L.H., Oros, J., Jacobson, E.R., Curry, S.S., and Klein, P.A. (1999). Association of herpesvirus with fibropapillomatosis of the green turtle Chelonia mydas and the loggerhead turtle Caretta caretta in Florida. Dis. Aquat. Organ. *37*, 89–97.
3. Quackenbush, S.L., Work, T.M., Balazs, G.H., Casey, R.N., Rovnak, J., Chaves, A., duToit, L., Baines, J.D., Parrish, C.R., Bowser, P.R., and Casey, J.W. (1998). Three closely related herpesviruses are associated with fibropapillomatosis in marine turtles. Virol. *246*, 392–399.
4. Herbst, L.H., Jacobson, E.R., Klein, P.A., Balazs, G.H., Moretti, R., Brown, T., and Sundberg, J.P. (1999). Comparative pathology and pathogenesis of spontaneous and experimentally induced fibropapillomas of green turtles (Chelonia mydas). Vet. Pathol. *36*, 551–564.
5. Herbst, L.H., Jacobson, E.R., Moretti, R., Brown, T., Sundberg, J.P., and Klein, P.A. (1995). Experimental transmission of green turtle fibropapillomatosis using cell-free tumor extracts. Dis. Aquat. Organ. *22*, 1–12.
6. Roizman, B., and Pellett, P.E. (2001). The family Herpesviridae: a brief introduction. In Fields Virology, Volume 2, 4th Edition, D.M. Knipe, P.M. Howley, D.E. Griffin, R.A. Lamb, M.A. Martin, B. Roizman and S.E. Straus, eds. (Philadelphia: Lippincott Williams & Wilkins), pp. 2381–2397.
7. Coberley, S.S., Condit, R.C., Herbst, L.H., and Klein, P.A. (2002). Identification and expression of immunogenic proteins of a disease-associated marine turtle herpesvirus. J. Virol. *76*, 10553–10558.
8. Nigro, O., Yu, G., Aguirre, A.A., and Lu, Y. (2004). Sequencing and characterization of the full-length gene encoding the single-stranded DNA binding protein of a novel Chelonian herpesvirus. Arch. Virol. *149*, 337–347.
9. Umene, K., and Sakaoka, H. (1999). Evolution of herpes simplex virus type 1 under herpesviral evolutionary processes. Arch. Virol. *144*, 637–656.
10. Davison, A.J. (2002). Evolution of the herpesviruses. Vet. Microbiol. *86*, 69–88.
11. McGeoch, D.J., Dolan, A., and Ralph, A.C. (2000). Toward a comprehensive phylogeny for mammalian and avian herpesviruses. J. Virol. *74*, 10401–10406.
12. Janke, A., Erpenbeck, D., Nilsson, M., and Arnason, U. (2001). The mitochondrial genomes of the iguana (*Iguana iguana*) and the caiman (*Caiman crocodylus*): implications for amniote phylogeny. Proc. R. Soc. Lond. B Biol .Sci. *268*, 623–631.
13. Muir, W.A., Nichols, R., and Breuer, J. (2002). Phylogenetic analysis of varicella–zoster virus: evidence of intercontinental spread of genotypes and recombination. J Virology *76*, 1971–1979.
14. Sakaoka, H., Kurita, K., Iida, Y., Takada, S., Umene, K., Kim, et al. (1994). Quantitative analysis of genomic polymorphism of herpes simplex virus type 1 strains from six countries: studies of molecular evolution and molecular epidemiology of the virus. J. Gen. Virol. *75*, 513–527.
15. Marko, P.B. (2002). Fossil calibration of molecular clocks and the divergence times of geminate species pairs separated by the Isthmus of Panama. Mol. Biol. Evol. *19*, 2005–2021.
16. Bowen, B.W., Nelson, W.S., and Avise, J.C. (1993). A molecular phylogeny for marine turtles: trait mapping, rate assessment, and conservation relevance. Proc. Natl. Acad. Sci. USA *90*, 5574–5577.

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