# Genetic diversification of field strains of bluetongue virus

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## Summary

The considerable genetic heterogeneity of field strains of bluetongue virus (BTV) occurs as a consequence of both genetic drift and shift. Comparison of strains of BTV from the People's Republic of China and North America showed that viruses from the two regions were readily distinguished by sequence analysis of their S10 (which encodes the NS3/3A proteins) but not their L2 (which encodes the serotype-specific VP2 protein) genes. Subsequent laboratory studies showed that individual BTV genes evolve through a combination of genetic drift coupled with founder effect in vector insects. This model explains the diversification of BTV gene segments within each region, and can be extrapolated to explain diversification of BTV into distinct topotypes worldwide.

## Keywords

Bluetongue virus – Evolution – Founder effect – Genetic drift – Quasispecies – Toptypes.

Genetic heterogeneity of field strains of bluetongue virus (BTV) occurs as a consequence of both genetic drift and shift. The consequence of these two phenomena is a remarkable heterogeneity amongst strains of BTV that circulate in endemic regions such as California, even amongst virus strains that co-circulate (1, 5, 6, 7, 9). Reassortment of BTV genes is responsible for genetic shifts amongst strains of BTV, and has been demonstrated after infection of either the ruminant host or insect vector with different strains or serotypes of BTV (10, 11). Accumulation of nucleotide substitutions within individual BTV genes leads to genetic drift of each.

Australian workers first proposed the term of virus 'topotypes' for the region-specific grouping of BTV strains that they observed after sequence analysis of the L3 gene of each virus (8). To further evaluate the region-specific grouping (topotype clustering) of field strains of BTV, we first compared the S10 and portions of the L2 genes of Chinese and North American strains of BTV (2). Phylogenetic analysis of the S10 gene segregated the Chinese viruses into a monophyletic group distinct from the American viruses, whereas analysis of the L2 gene segregated strains of BTV according only to serotype, regardless of geographic origin. These studies showed not only that BTV genes evolve independently of one another, but also confirmed that BTV strains from distinct geographic locations can be classified as topotypes based on the sequence of a conserved gene that assigns a virus isolate to a specific geographic region, regardless of serotype.

In subsequent studies to further characterise the genetic diversity of field strains of BTV that co-circulate at a single site, we directly amplified and sequenced the S10 gene of field strains of BTV contained within *Culicoides sonorensis* (*C. sonorensis*) collected from a dairy in southern California (4). Phylogenetic analysis established that the S10 gene of BTV in *C. sonorensis* collected from the site existed as a heterogeneous but related population, probably arising from genetic drift. Thus, we hypothesised that viral genes undergo genetic drift during alternating passage of BTV in its ruminant and insect hosts. To test this hypothesis, variation in the consensus sequence and quasispecies heterogeneity of the L2 and S10 genes of BTV was determined during alternating infection of a sheep and calf with BTV that was transmitted by *C. sonorensis* (3). This study demonstrated that individual BTV gene segments evolve independently of one another by genetic drift in a host-specific fashion, generating quasispecies populations in the ruminant and insect hosts. A unique viral variant randomly ingested by *C. sonorensis* that fed on the viraemic sheep resulted in fixation of a novel genotype, thereby demonstrating founder effect. Therefore, genetic drift coupled with founder effect offers a model for the diversification

of BTV gene segments at a single site, and can be extrapolated to explain diversification of BTV into distinct topotypes worldwide (1).

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