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Towards inferring the global movement of *beak and feather disease virus*

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ABSTRACT

Beak and feather disease virus (BFDV) is a circular single-stranded DNA virus that causes psittacine beak and feather disease. We analysed 184 publically available BFDV full genomes to infer both the approximate geographical origin of the most recent common ancestor (MRCA) of these sequences and past BFDV long-range migrations using a Bayesian phylogeographic analyses. While the analysed BFDV sequences were sampled over too brief a period to ensure a strong enough temporal signal for accurate long-term substitution rate estimation, we were nevertheless able to identify Australia as the most likely location of the MRCA; A finding consistent with historical records of BFDV incidence. We additionally identified various trans-global BFDV movements including a number from Europe to regions of the world where psittacines are naturally found. This is concerning because it suggests that any BFDV variants that might emerge in captive European birds could directly threaten wild psittacine populations.

Introduction

Beak and feather disease virus (BFDV) causes the highly infectious and often fatal, psittacine beak and feather disease (PBFD) that infects over 60 different psittacine species. Among these species are several that are at risk of extinction including the South African Cape Parrot (*Poicephalus robustus*), and the Echo Parakeet (*Psittacula echo*) (IUCN Red List of Threatened species, version 2011, http://www.iucnredlist.org/).

BFDV is a circular single-stranded DNA (ssDNA) virus belonging to the family *Circoviridae*. The viral genome is bi-directionally transcribed and encodes at least two proteins; a replication associated protein (Rep) expressed from the virion strand and a capsid protein (CP) expressed from the complementary strand (which is present within a double-stranded replicative intermediate).

Since it was officially described in a number of Australian psittacine birds in the 1970s (Pass and Perry, 1984), incidences of PBFD have subsequently been reported in various countries across Europe, Asia, North America and Africa and on several islands in the Pacific and Indian Oceans.

Transmission is thought to include both horizontal and vertical modalities, the former through shedding of virus particles in feather dust, crop secretions, or faeces (Ritchie et al., 1991), and the latter from infected birds to embryonated eggs (Rahaus et al., 2008). Within individual wild-parrot flocks, incidences of BFDV can be higher than 41%, which suggests that it can be highly infectious and that horizontal transmission is likely the major route of transmission under natural conditions (Raidal et al., 1993). In the context of captive environments such as breeding facilities that supply psittacine birds (such as cockatoos, parrots, parakeets, and lovebirds) for the pet trade, horizontal transmission through environmental contamination is also probably the most prevalent mode of infection.

Infection may be acute or chronic and typical PBFD symptoms include anaemia, depression and lethargy which ultimately lead to abnormal feather growth culminating in feather loss (Ritchie et al., 1989b). In severe cases, beak and claw deformities may be displayed (Pass and Perry, 1984). Symptoms may however be species-specific as beak and claw involvement appears to be more prevalent in cockatoos than in other psittacine species (McOrist et al., 1984; Ritchie et al., 1989b). Possible beak deformities include elongation, transverse or longitudinal cracking, and palatine necrosis (McOrist et al., 1984; Pass and Perry, 1984; Ritchie et al., 1989a, 1989b). PBFD can present as a peracute, acute or chronic disease. Younger birds, neonates to fledglings, tend to suffer from

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the peracute and acute forms, in which sudden death can occur with no (peracute) or mild (acute) feather dystrophy (Doneley, 2003; Ritchie et al., 1989a). The prevalence of the chronic form is higher in older birds and is not always fatal with infected birds potentially surviving for many years (Ritchie et al., 1989b). However, since the disease results in immune suppression, chronically infected birds often eventually succumb to secondary infections (Latimer et al., 1991; Ritchie et al., 1989b; Todd, 2000).

Although BFDV has an almost global distribution and is found in both wild and captive bird populations (see Julian et al., 2012, 2013; Kundu et al., 2012; Massaro et al., 2012), circumstantial evidence indicates that it may have originated in Australia (Pass and Perry, 1984) and spread to the rest of the world in modern times. Although the diversity of wild psittacine species in the Afro-Asian and Neo-tropical regions of the world remain largely under-represented in this analysis, it likely equals that of parrot diversity in Australasia (Forshaw, 2010). The Australian BFDV origin hypothesis is plausible for several reasons in addition to the fact that the diversity of wild psittacine species is relatively high in Australasia. For example, it is also notable that what is perhaps the first recorded description of PBFD occurred in a letter to an Australian journal in 1907, in which the author notes a personal observation from 1887 of wild Red-rumped Parrots (*Psephotus haematonotus*) in the Adelaide hills being unable to fly due to a complete loss of feathers (Ashby, 1907). Another probable early reported case of a PBFD infection was that of a captive Sulphur-crested Cockatoo in Sydney, named Cocky Bennett, a bird so well-known locally that his death in 1916 at the estimated age of 120 years old (which is surprising given that it was likely infected by BFDV) warranted an obituary in the local newspaper (Centre for Fortean Zoology Australia, 2011). Descriptions and photographs of Cocky from the early 1900s indicate that he was mostly featherless and had a severely elongated upper beak (Nicholls, 1914), both of which are characteristic symptoms of PBFD. The origin of Cocky is uncertain and since *Cacatua galerita* were rare in the Sydney basin at that time, it is likely that he originated in the Moluccas (Forshaw, 2010) where this species was much more common.

As part of the aviculture and pet trade many parrots species have been trafficked around the world and in some cases these have established wild populations following escape or release from captivity (Bull, 1973; Butler, 2005). In Europe where there are no native psittacines, wild exotic populations of these birds are now found the many of temperate regions (Chiron et al., 2009; Muñoz and Real, 2006; Strubbe and Matthysen, 2009) and account for approximately 18% of Europe's wild populations of exotic birds (Strubbe and Matthysen, 2009). As a result of this, in 2007 the import and trade of wild-caught exotic parrots in Europe was banned (Commission Regulation (EC) no. 318/2007).

Whereas identifying when and where viruses like BFDV first arose can yield valuable insights into how such pathogens emerge, the identification of virus movement pathways and the estimation of virus movement rates can provide crucial information for both future disease forecasting efforts, and the formulation of governmental policies aimed at restricting the transport of avian viruses across national borders. For example, by identifying both key historical movement routes and the locations where the most important contemporary BFDV lineages first arose, it may be possible to impose modest sanitation measures and restrictions on future movements of psittacine birds in a targeted approach, leading to significantly reduced risks of spreading newly emergent recombinant BFDV strains or other avian pathogens around the world.

Here we use all currently available BFDV genome sequence data from around the world to both infer the approximate geographical region where BFDV originated, and identify at least some of the historical global movements that have enabled this virus to achieve its present global distribution.

Results and discussion

For the full genome dataset the general time reversible model with gamma distributed rate variation and a proportion of invariable sites (GTR+G+I) was identified as the best-fit nucleotide substitution model. Similarly, the uncorrelated lognormal relaxed-clock model invariably fitted the data better than the strict-clock model under all three of the demographic models tested (constant population size, exponential population growth and the Bayesian skyline plot). However, none of the demographic models fitted the data better than any other under the uncorrelated lognormal relaxed-clock model.

As the choice of coalescent prior has been shown to have a negligible effect on estimates of viral movement dynamics using these discretized phylogeographic diffusion models (Lemey et al., 2009) we chose to employ the constant population size tree prior under the uncorrelated lognormal relaxed-clock model to reconstruct the spatiotemporal dynamics and evolution of BFDV using BEAST (Drummond and Rambaut, 2007).

Specifically, for each of the RF, *rep* and *cp* datasets ten independent replicate runs of the Markov chain, of between 2×10^8 (RF) and 4×10^8 (*cp* and *rep*) steps in length were performed to produce posterior distributions of trees containing at least 5000 genealogies. When independent replicates converged to the same overall tree-likelihood space the log and tree files were combined using LogCombiner (Drummond and Rambaut, 2007) to ensure that effective sample size (ESS) estimates for all model parameters were always >200. Maximum clade credibility (MCC) trees for each dataset were determined from the complete posterior distribution of trees produced from these runs and represented the point estimate of the tree with the highest cumulative posterior probability support amongst all the trees in the posterior distribution of trees.

The MCC trees produced from the discrete model phylogeographic analyses carried out on these trees for the RF, *rep* and *cp* datasets are presented in Figs. 1 and 2 and Supplementary Fig. 1. The RF MCC tree had the highest overall statistical support (Fig. 1) and although the topologies of the RF and *rep* MCC trees are similar in many respects, notable differences are apparent. For example, in the *rep* tree (Fig. 2) a monophyletic clade containing sequences sampled between 2000 and 2011 that comprised three sub-clades monophyletic by sampling location (Australia, Thailand and New Caledonia) branches off the root whereas, in the RF tree (Fig. 1), two Australian sequences sampled in 2000 (captive) and 2009 (wild) from rainbow lorikeets form a basal clade distinct from the remaining sequences. Support for these alternative groupings of the basal branches was however, not high (*rep*=0.59, RF=0.61). In contrast to both the RF and *rep* trees the *cp* tree (Supplementary Fig. 1) had a very different topology with most basal branches occupied by sequences collected in Poland and Japan.

Despite these topological differences between the various MCC trees there were commonalities among them such as the high proportion of clades containing New Zealand and New Caledonia sequences that were both strongly supported and monophyletic by location. It is notable that the New Caledonian sequences from *Trichoglossus haematodus* (deplanchii) and the New Zealand sequences from *Cyanoramphus auriceps*, *Cyanoramphus novaezelandiae*, and *Platycercus eximius* were all sampled from wild birds. The fact that relatively genetically uniform BFDV populations are present within these geographically isolated wild-bird populations suggests that BFDV transmissions to these populations of divergent virus lineages may not occur very regularly. It should be stressed, however, that BFDV incidences in these bird populations were low (11–23%; Julian et al., 2012; Massaro et al., 2012) and the populations have not been longitudinally studied. It is plausible