GENETIC DIVERSITY OF PSITTACINE BEAK AND FEATHER DISEASE VIRUSES: IMPLICATIONS FOR THEIR ORIGIN AND PATHOGENICITY

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INTRODUCTION

The psittacine beak and feather disease virus (PBFDV) occurs around the world (Bassami et al., 2001; Health et al., 2004; Hsu C-M et al., 2006; Katoh et al., 2010; Ortiz-Catedral et al., 2010). Its dissemination has undoubtedly been the result of movement of birds through the pet trade but is also likely to have been the result of natural spread of the virus in parrots prior to the development of international commerce in birds. Genetically, PBFDV is a complex virus that has apparently evolved with different species of parrots for an extended period of time. Determining the origins of the various genotypes of the PBFDV is difficult as it is likely that different genotypes are able to infect many species and once a virus is introduced into a mixed collection of parrots, it may end up infecting species with which it did not co-evolve.

Despite the challenges of correlating genotypes with species, a picture is arising that suggests there are at least three major PBFDV clades with many subclades within these genotypes. The three major clades are the African, cockatoo, and lorikeet (Raue et al.,2004; de Kloet and de Kloet,2004; Heath et al.,2004; Khalasi et al.,2005; Kondia et al., 2006; Ritchie et al., 2003;Varsani et al., 2010). The PBFDVs infecting budgerigars may represent a subclade of the cockatoo clade or be considered to be its own clade (Varsani et al., 2010).

The purposes of this study were to begin to determine if:

1. there are specific genotypes present in specific species of parrot in the wild in Australia;
2. these genotypes and the others are present in avicultural species in Australia; and
3. specific genotypes remain within species in aviculture or freely mix between species.

MATERIALS AND METHODS

Sample collection and preparation. Samples of growing feathers or blood were collected from 26 psittacine birds that had feather lesions consistent with PBFD (Table 1). DNA was extracted from an approximately 3mm section of feather calamus using DNeasy tissue kits (QIAGEN Doncaster, Victoria, AU). Eluted DNA was stored at -20 ^{o}C until use.

Amplification of PBFDV DNA. Primers (Table 2) were used with PCR to amplify the open reading

frame of the C1 capsid protein (nt 1228 to 1977). PCR reactions were performed in 20 µL volumes containing 1.5 µL $MgCl_2$, 0.4 µL dNTP and 0.12 µL of Taq DNA polymerase and the buffer supplied by the manufacturer (all reagents Promega, Madison, WI, USA). Amplification was done in a Corbett Gradient Palm–Cycler™ Thermocycler (Corbett Research, Montlake, NSW, AU). Samples were initially incubated at 94°C, and then amplified with 20 cycles with an annealing temperature of 58°C for 30 seconds, an extension temperature of 72°C for 1 minute, and a melting temperature of 94°C for 30 seconds. Amplification products were separated on an agarose gel containing ethidium bromide and visualized under ultraviolet light. Amplicons were purified using Microcon® Centrifugal Filter Units (Millipore, Billerica, MA, USA) and sequenced in both directions using a commercial laboratory (Australian Genome Research Facility Ltd, Westmead, NSW, AU).

Phylogenetic analysis. Sequences were uploaded onto and corrected and aligned with CLC Main Workbench DNA Analysis Program (Mountainview Estate, Queensland, AU). Phylogenetic analysis was done using the same program using neighbour-joining (NJ) and bootstrap analysis (Saitou and Nei 1987) using our new DNA sequences and sequences obtained from Genbank (Table 2). Phylogenetic trees were transferred onto MEGA 4.0 DNA software (Kumar et al., 2008) for annotation.

RESULTS

Twenty six new sequences and 33 previous published sequences were used to produce a neighbour-joining phylogenetic tree (Figure 1). Two large clades were identified within which there were 12 smaller subclades. Subclades I, II, III, IV, X, XI and XII corresponded to the African genotype with sequences of lovebird origin clustering in subclades I and II and sequences from *Poicephalis* species clustering in group III and sequences of African grey parrot origin clustering in subclades X, XI, and XII. Subclades V, VI, VII, VIII, and IX formed the Australian clade. The bulk of sulphur-crested cockatoos mapped to subclade V, subclade VII was the lorikeet group, and subclade IX contained the majority of the sequences from galahs. Sequences of eclectus parrot origin were spread evenly over the African and Australian subclades as were sequences derived from Neotropical parrots. Lorikeets were also found to be infected with cockatoo and African strains as well as lorikeet strains. Three eclectus parrots, 2 lorikeets, 1 African grey parrot, 1 scarlet macaw and 1 lovebird that were from Australian sources where infected with African genotypes. None of the genotypes from wild Australian parrots were African genotypes.

DISCUSSION

Results of this study reinforce what other investigators have shown and that are there is a least two major genotypes of the PBFDV, the African and the Australian genotypes. Within the Australian genotype there is a cockatoo subgenotype that is predominately found in wild and captive sulphur-crested cockatoos and possibly other white cockatoos. A second subgenotype corresponds to the previously recognized lorikeet genotype. The third subgenotype is most commonly found in galahs. The African genotype can also be divided into the African grey, lovebird and *Poicephalus* spp subgenotypes.

African subgenotypes are present in Australia in avicultural species, but have not been identified in wild parrots at this point of the study. It is likely that these genotypes entered Australia as the result of either the legal or illegal pet trade. This study also demonstrates what others have suggested and that is while some genotypes are more likely to occur in certain species both in the wild and in captivity, they are capable of infecting and causing disease in other species as well.

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REFERENCES

Bassami MR, Ypelaar I, Berryman D, Wilcox GE, Raidal SR (2001). Genetic diversity of beak and feather disease virus detected in psittacine species in Australia. *Virology*. **279**:392-400.

de Kloet E, de Kloet SR (2004). Analysis of the beak and feather disease viral genome indicates the existence of several genotypes which have a complex psittacine host specificity. *Archives of Virology*. **149**:2393-412.

Heath L, Martin DP, Warburton L, Perrin M, Horsfield W, Kingsley C, Rybicki EP, Williamson A-L (2004). Evidence of unique genotypes of beak and feather disease virus in southern Africa. *Journal of Virology*. **78**:9277-9284.

Hsu C-M, Ko C-Y, Tsai H-J (2006). Detection and sequence analysis of avian polyomavirus and psittacine beak and feather disease virus from psittacine birds in taiwan. *Avian Diseases*. **50**:348-353.

Katoh H, Ohya K, Ise K, Fukushi H (2010). Genetic analysis of beak and feather disease virus derived from a cockatiel (*Nymphicus hollandicus*) in Japan. *The Journal of Veterinary Medical Science.* **72**: 631–634.

Khalesi B, Bonne N, Stewart M, Sharp M, Raidal R (2005). A comparison of haemagglutination, haemagglutination inhibition and PCR for the detection of psittacine beak and feather disease virus infection and a comparison of isolates obtained from loriids. *Journal of General Virology*. **86**:3039-3046.

Kondiah K, Albertyn J, Bragg RR (2006). Genetic diversity of the Rep gene of beak and feather disease virus in South Africa. *Archives of Virology*. **151**: 2539-45.

Kumar S, Nei, M, Dudley J, Tamura K (2008). MEGA: A biologist-centric software for evolutionary analysis of DNA and protein sequences. *Briefings Bioinformatics*. **9**:299-306.

Ortiz-Catedral L, Kurenbach, B, • Melanie Massaro M, McInnes K, Brunton DH, Hauber ME, Martin DP, Varsani A (2010). A new isolate of beak and feather disease virus from endemic wild red-fronted parakeets (*Cyanoramphus novaezelandiae*)
in New Zealand. *Archives of Virology*. **155**:613–620.

Raue R, Johne R, Crosta L, Burkle M, Gerlach H, Müller H. (2004). Nucleotide sequence analysis of a C1 gene fragment of psittacine beak and feather disease virus amplified by real-time polymerase chain reaction indicates a possible existence of genotypes. *Avian Pathology*. **33**:41-50.

Ritchie PA, Anderson IL, Lambert D M (2003). Evidence for specificity of psittacine beak and feather disease viruses among avian hosts. *Virology*. **306**: 109-15.

Saitou N, Nei M (1987). The neighbor-joining method: a new method for reconstructing phylogenetic

trees. *Molecular Biology and Evolution.*. **4**:406-25.

Varsani A, de Villiers GK, Regnard GL, Bragg RR, Kondiah K, Hitzeroth II, Rybicki EP(2010). A unique isolate of beak and feather disease virus isolated from budgerigars (*Melopsittacus undulatus*) in South Africa. *Archives of Virology*. **155**:435–439.

Table 1. Species of origin and genotype of sequences derived from Australian parrots

Common Name	Scientific Name	Genotype
Sulphur-crested cockatoo	*Cacatua galerita*	V
Sulphur-crested cockatoo	*Cacatua galerita*	V
Sulphur-crested cockatoo	*Cacatua galerita*	V
Sulphur-crested cockatoo	*Cacatua galerita*	V
Sulphur-crested cockatoo	*Cacatua galerita*	V
Sulphur-crested cockatoo	*Cacatua galerita*	V
Sulphur-crested cockatoo	*Cacatua galerita*	V
Sulphur-crested cockatoo	*Cacatua galerita*	V
Cockatoo	*Cacatua sp.*	V
Cockatoo	*Cacatua sp.*	V
Major Mitchell's cockatoo	*Lophochroa leadbeateri*	V
Major Mitchell's cockatoo	*Lophochroa leadbeateri*	V
Rainbow lorikeets	*Trichoglossus haematodus*	V
Rainbow lorikeets	*Trichoglossus haematodus*	VII
Rainbow lorikeets	*Trichoglossus haematodus*	VI
Rainbow lorikeets	*Trichoglossus haematodus*	VI
Rainbow lorikeets	*Trichoglossus haematodus*	XI
Galah	*Eolophus roseicapilla*	IX
Galah	*Eolophus roseicapilla*	V
Galah/Corella	*Eolophus roseicapilla*	IX
Galah/Corella	*Eolophus roseicapilla*	IX
Eclectus parrots	*Eclectus roratus*	XI

Common Name	Scientific Name	Genotype
Eclectus parrots	*Eclectus roratus*	I
Eclectus parrots	*Eclectus roratus*	XI
Eclectus parrots	*Eclectus roratus*	V
Lovebird	*Agapornis sp.*	I

Table 2. Primers used to amplify the complete nucleotide of captive protein gene sequences of psittacine species (Heath et al., 2004).

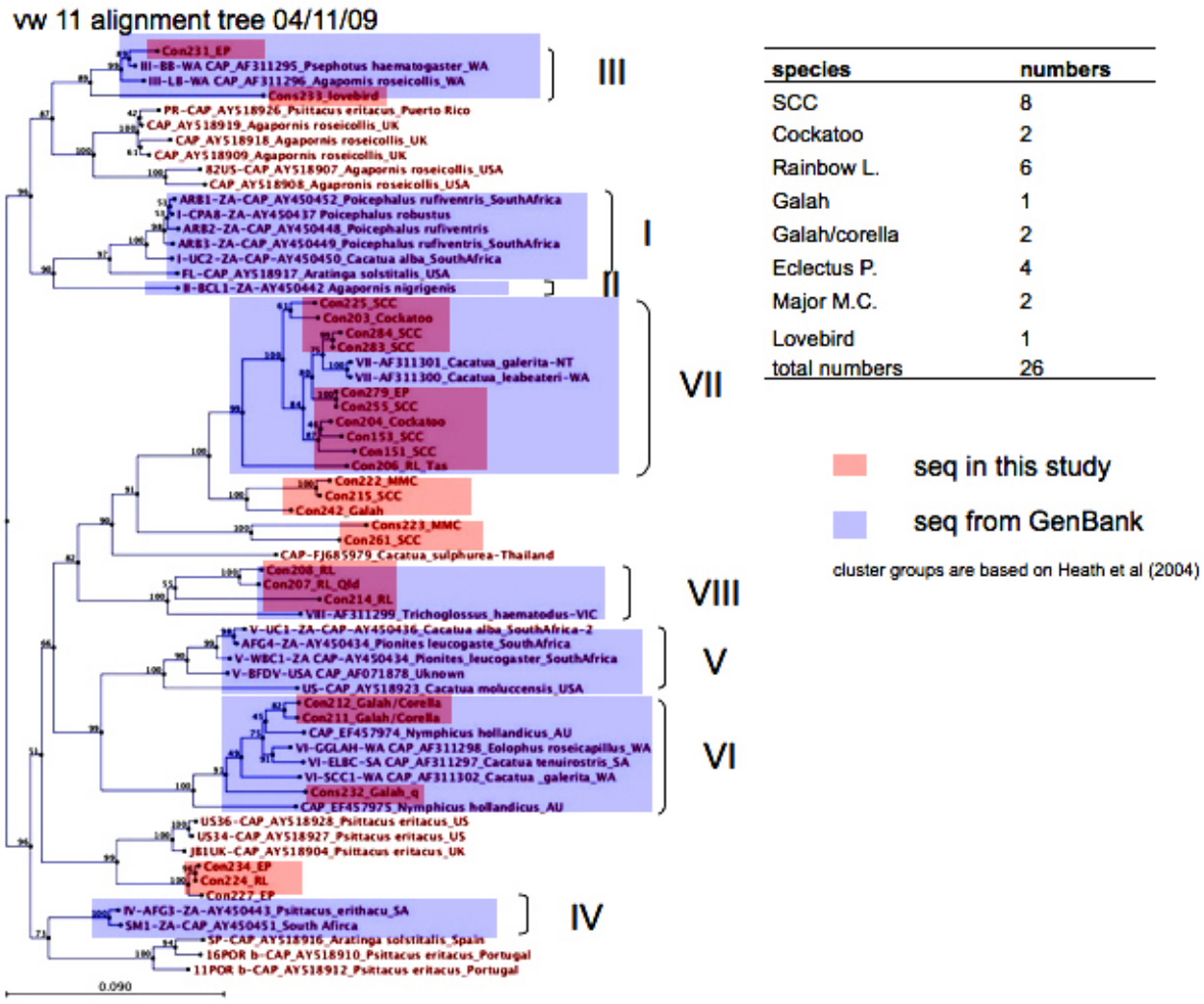
Primer	Sequence :(5'-3')	Size	Position in virus strain
CP forward	GCGGCCGCATGCTGTGGGGCACCTCTAACTGC	32	1228-1258
CP reverse	CTCGAGTCTTTATTAAGTACTGGGATTG	28	1951-1977

Table 3. Abbreviation, GenBank accession number, and host species for BFDV sequences used in the phylogenetic analysis.

Abbreviation	GenBank Accession No.	Host species	Common name
BB-AUS	AF311295	*Psephotus haematogaster*	Blue bonnet parrot
LB-AUS	AF311296	*Agapornis roseicollis*	Peach faced lovebird
ELBC-AUS	AF311297	*Cacatua tenuirostris*	Long billed corella
ER-AUS	AF311298	*Eolophus roseicapillus*	Galah
TH-AUS	AF311299	*Trichoglossus haematodus*	Rainbow lorikeet
MMC-AUS	AF311300	*C. leadbeateri*	Major Mitchell cockatoo
SCC-NT	AF311301	*C. galerita*	Sulphur crested cockatoo
SCC2-AUS	AF311302	*C. galerita*	Sulphur crested cockatoo
SCC3-AUS	AF080560	*C. galerita*	Sulphur crested cockatoo
BFDV-USA	AF071878	*unknown*	Pooled virus
AFG3-ZA	AY450443	*Psittacus erithacus*	Africa grey parrot
AFG4-ZA	AY450434	*Psittacus erithacus*	Africa grey parrot
UC1-ZA	AY450436	*C. alba*	Umbrella cockatoo

Abbreviation	GenBank Accession No.	Host species	Common name
UC2-ZA	AY450450	*C. alba*	Umbrella cockatoo
WBC1-ZA	AY450434	*Pionites legucogaster*	White-bellied caique
SM1-ZA	AY450451	*Ara macao*	Scarlet macaw
ARB1-ZA	AY450452	*Poicephalus rufiventris*	African red-bellied parrot
ARB2-ZA	AY450448	*Poicephalus rufiventris*	African red-bellied parrot
ARB3-ZA	AY450449	*Poicephalus rufiventris*	African red-bellied parrot
CPA8-ZA	AY450437	*Poicephalus robustus*	Cape parrot
BCL1-ZAM	AY450442	*Agapornis nigrigenis*	Black-cheeked lovebird
AR7	AY518908	*Agapornis roseicollis*	Peach faced lovebird
AR8	AY518907	*Agapornis roseicollis*	Peach faced lovebird
PEP11	AY518912	*Psittacus erithacus*	Africa grey parrot
PEP16	AY518910	*Psittacus erithacus*	Africa grey parrot
ERF1	AY518917	*Eclectus roratus*	Eclectus parrot
CM	AY518923	*C. moluccensis*	Salmon-crested Cockatoo
PEPR	AY518926	*Psittacus erithacus*	Africa grey parrot
PE3-4	AY518927	*Psittacus erithacus*	Africa grey parrot
PE3-6	AY518928	*Psittacus erithacus*	Africa grey parrot
PEU-1	AY518904	*Psittacus erithacus*	Africa grey parrot
05-106	EF457974	*Nymphicus hollandicus*	Cockatiel
05-726	EF457975	*Nymphicus hollandicus*	Cockatiel

Figure 1. Neighbour joining tree of the putative capsid protein gene nucleotide sequences. Numbers at the nodes indicate bootstrap support. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The blue areas indicate the clusters of sequences used to define the BFDV lineage. The name of the subclades are CT (cockatoos) and LK (lorikeets), respectively. This Figure will be distributed at the conference.



species	numbers
SCC	8
Cockatoo	2
Rainbow L.	6
Galah	1
Galah/corella	2
Eclectus P.	4
Major M.C.	2
Lovebird	1
total numbers	26

Note: This page was inserted after the conference - the conference paper ends at page 74. Call this page 74a -Editor.