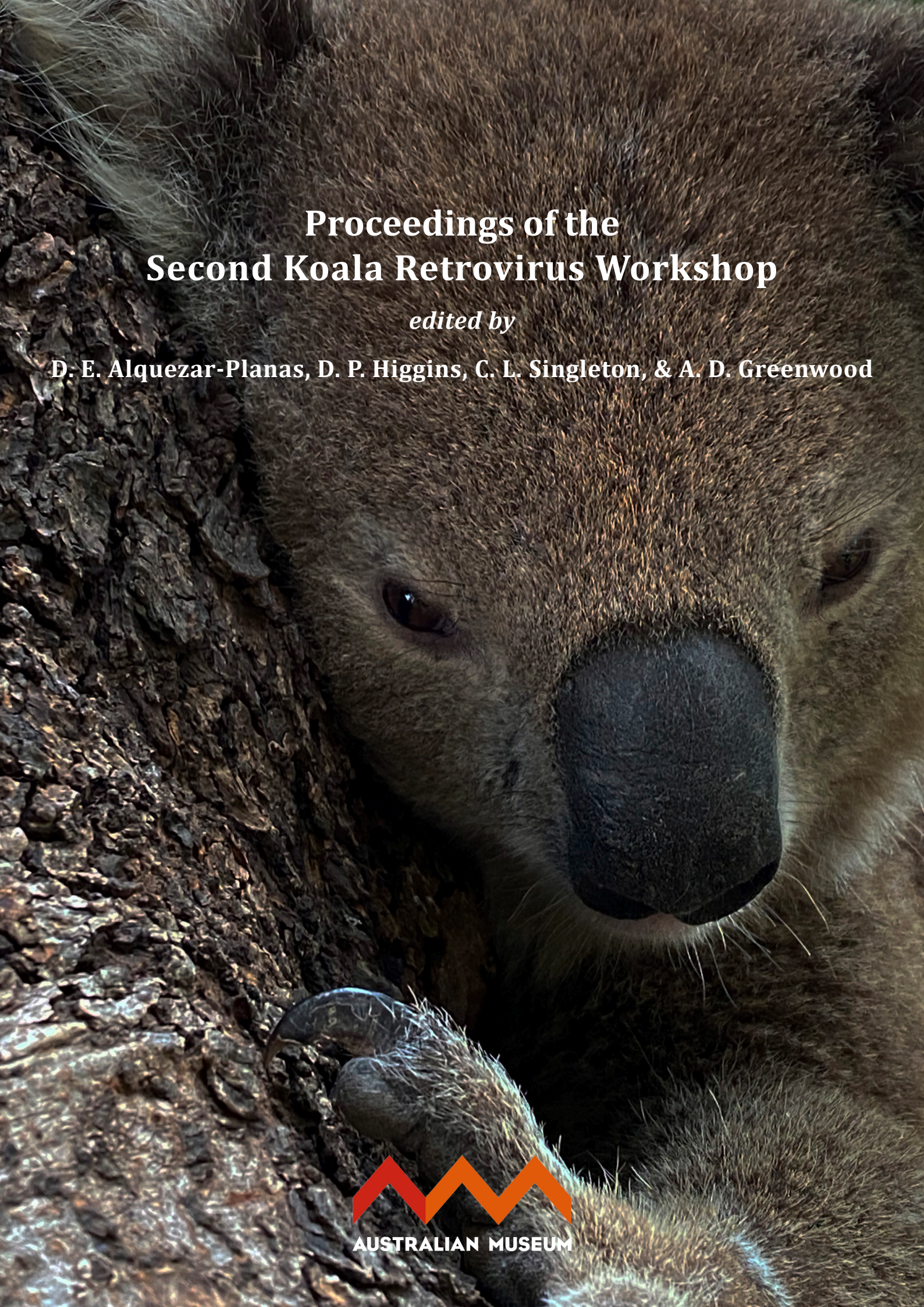
Proceedings of the Second Koala Retrovirus Workshop

edited by

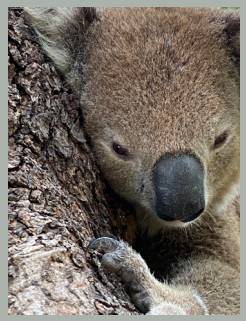
D. E. Alquezar-Planas, D. P. Higgins, C. L. Singleton, & A. D. Greenwood



AUSTRALIAN MUSEUM

Technical Reports of the Australian Museum Online **no. 38**

Table of Contents



Cover photo
by Damien P. Higgins

A series of peer-reviewed papers, edited by David E. Alquezar-Planas, Damien P. Higgins, Cora L. Singleton, & Alex D. Greenwood, and a discussion summary, from the *Second Koala Retrovirus Workshop* held online, 25–27 May 2021. Published 21 June 2023, in *Technical Reports of the Australian Museum Online* number 38, ISSN 1835-4211 (online).

The Australian Museum is a statutory authority of, and principally funded by, the NSW State Government.

Tech. Rep. Aust. Mus. Online
Number 38, pp. 3–6, 2023
https://doi.org/10.3853/j.1835-4211.38.2023.1831

Technical Reports of the Australian Museum Online
a peer-reviewed open-access journal
published by the Australian Museum, Sydney
communicating knowledge derived from our collections
ISSN 1835-4211 (online)

An Overview of Koala Retrovirus Epidemiology in Australia

Rachael Tarlinton

School of Veterinary Medicine and Science, University of Nottingham,
United Kingdom

Abstract. Koala retrovirus (KoRV) epidemiology varies across koala (*Phascolarctos cinereus*) populations with distinct differences in viral prevalence, sequence diversity, and disease impact. Curiously the more genetically restricted southern populations are less impacted by KoRV with the virus not endogenized in its replication competent form in these animals. These southern animals do, however, have replication defective recKoRV variants in their genomes indicating historical exposure to KoRV and recKoRV. Whether southern animals are inherently resistant to KoRV infection and endogenization is not clear. It is also not clear whether the current regional epidemiological patterns will persist or whether exposure to animals with infectious KoRV or cross-breeding between different genetic populations will change the KoRV prevalence with time.

Introduction

Both koala (*Phascolarctos cinereus*) genetics and koala retrovirus (KoRV) prevalence vary regionally across Australia, with a stark demarcation between a more genetically diverse "northern" group (New South Wales and Queensland) and a genetically restricted "southern" group (Victoria and South Australia). These groups of animals also display markedly different disease profiles, with putatively KoRV-related disease syndromes at a much lower rate in the southern animals. All northern animals ever studied have endogenous KoRV-A alongside varying prevalence of other KoRV genotypes that do not appear to be endogenous. Endogenous KoRV loci are shared amongst closely related individuals but are not fixed across the species. Northern koalas also have multiple copies of defective KoRV variants in their genomes, where the central portion of the KoRV genome has been replaced by another koala retro-element termed Phascolarctid endogenous retroelement (PhER). These are known as recKoRVs and are also not fixed.

The southern animals were re-established from off-shore island colonies after localized extinction in the 1920's with a marked genetic bottleneck evident. Southern animals display varying KoRV prevalence without endogenous KoRV loci. Those animals that are KoRV positive tend to have lower viral loads than their northern counterparts. It was previously thought that many of these animals were KoRV free; however, recent work has demonstrated that many (perhaps all) animals that test negative for the KoRV *pol* gene PCR (the most used diagnostic for all KoRV variants) have recKoRV variants within their genomes. These are distinct from the recKoRV variants in the northern animals with an additional indel of unidentified DNA between the KoRV *gag* and PhER sequences. It is not clear at this stage what the significance of this is for potential to cause disease. It is possible that the presence of these variants inhibits infectious KoRV (as happens with defective endogenous retroviruses in other species). It is also possible that southern animals are simply not born tolerized to KoRV-A (or other variants) and are better able to control virus replication via their

Keywords: koala retrovirus, KoRV, Phascolarctid endogenous retroelement
ORCID iD: Rachael E. Tarlinton https://orcid.org/0000-0003-3325-2311
Corresponding author: Rachael E. Tarlinton rachael.tarlinton@nottingham.ac.uk
Submitted: 5 January 2021 **Accepted**: 5 May 2023 **Published**: 21 June 2023 (online only)
Publisher: The Australian Museum, Sydney, Australia (a statutory authority of, and principally funded by, the NSW State Government)
Citation: Tarlinton, Rachael E. 2023. An overview of koala retrovirus epidemiology in Australia. In *Proceedings of the Second Koala Retrovirus Workshop*, ed. D. E. Alquezar-Planas, D. P. Higgins, C. L. Singleton, and A. D. Greenwood. *Technical Reports of the Australian Museum Online* 38: 3–6. https://doi.org/10.3853/j.1835-4211.38.2023.1831

immune responses. Ancestors of the founder populations of the southern animals must have been infected with KoRV at some stage to have accumulated recKoRVs in their genomes but why KoRV-A is not also endogenous in these animals or what the implications are for cross-breeding of animals at border areas between populations is still unknown.

Discussion

Koala retrovirus is an unusual pathogen in that it is currently undergoing the transition between being an infectious transmissible virus and a retrotransposon carried by its host's genome (Tarlinton *et al*., 2006). Retroviruses are single stranded RNA viruses that make a DNA copy of themselves that is integrated in the host cell's DNA as part of their lifecycle. If this copy is integrated into a germ line cell (sperm, ova or progenitor of these in early stage zygotes) it becomes inherited. This process is surprisingly common with all vertebrates to date having multiple endogenous retroviruses integrated into their genomes. However, most have become attenuated with time, accumulating mutations that render them non-functional as a virus (Symer & Boeke, 2010). The process of re-integration into the genome can continue for some time after the original infectious virus becomes extinct, with the retroviral genomic copies forming a "fossil" record of a host's past viral exposure in evolutionary history.

KoRV is one of a small group of viruses that have both infectious forms currently circulating and accumulated host germline copies of the virus. This greatly complicates assigning attribution for disease pathogenesis in populations where animals are born with inherited germline copies of the virus. Though it is now apparent that both the accumulation of new somatic insertions of KoRV and the inheritance of viral insertions near or in oncogenes is the trigger for the very high rates of haematopoietic neoplasia seen in koalas (McEwen *et al*., 2021).

Koala populations have been through multiple bottlenecks with a marked population contraction approximately 30–40,000 years ago. Several distinct geographical barriers are evident in studies of koala population genetics with five distinct geographical clusters: North Queensland, South East Queensland, Mid-North Coast New South Wales, South Coast New South Wales and Victoria/South Australia (Johnson *et al*., 2018). The most recent and dramatic genetic segregation was the bottleneck in the southern population induced by hunting pressures upon European colonization. Most of the southern population was effectively extinct by approximately 1920, and this region was repopulated from a very small number of animals that had been removed to offshore island sanctuaries (Ruiz-Rodriguez *et al*., 2016). Consequently, the southern (Victoria/South Australia) population has a markedly lower genetic diversity than the other populations (Johnson *et al*., 2018; Ruiz-Rodriguez *et al*., 2016; Neaves *et al*., 2016).

This split in general koala conservation genetics is also evident in the distribution of their koala retrovirus complement, with marked differences evident between northern and southern koala populations (Sarker *et al*., 2019, 2020) as well as structuring of retroviral diversity at local population levels in the northern animals (Quigley *et al*., 2018). To date, all animals in the northern populations have the originally described variant of koala retrovirus known as KoRV-A, thought to be the endogenous variant with an attenuated CETAG envelope (*env*) protein motif (Quigley *et al*., 2018, 2021a) along with a defective variant of this strain with a frameshift mutation and stop codon in *env* (Quigley *et al*., 2021b). Diagnostic tests used for KoRV are usually PCR or qPCR based tests designed to detect KoRV polymerase (*pol*) or *env* genes (Tarlinton *et al*., 2005; Stephenson *et al*., 2021). Many animals in southern populations do not have KoRV based on these tests. KoRV-A is detected in some southern animals but at a rate and copy number per genome equivalent in individuals that implies it is solely exogenous (Speight *et al*., 2020; Legione *et al*., 2016). This is further supported by the increased prevalence (in animals that test positive for KoRV) of the presumed exogenous variant of KoRV-A with the more virulent CETAG motif (Quigley *et al*., 2021b).

Other strains of KoRV, based on sequencing of the hypervariable region of the surface unit of the *env* gene, have been described (Legione *et al*., 2016). These have never been detected without concurrent detection of KoRV-A, and it is not clear whether they circulate independently of KoRV-A or not (Sarker *et al*., 2019; Quigley *et al*., 2021b; Joyce *et al*., 2021). These also vary locally in different populations, with a general decrease in viral diversity and load evident further south in the koala population range (Sarker *et al*., 2019; McEwen *et al*., 2021). There has been much speculation as to whether these variants, particularly the B variant, are associated with increased virulence or differences in disease prevalence (Zheng *et al*., 2020; Xu *et al*., 2013; Waugh *et al*., 2017) but this has not been borne out in all studies (Quigley *et al*., 2019; Robbins *et al*., 2020).

The emerging picture from many groups' work on KoRV variants and prevalence is one of a distinct split between Victoria/South Australia animals and northern animals, with KoRV present in both endogenous and exogenous forms in the northern koalas but as an exogenous virus with reduced diversity in the southern animals. This coincides with different disease prevalence rates between these populations, with both neoplasia and clinical chlamydial disease at much lower rates in southern populations (Sarker *et al*., 2020; Quigley *et al*., 2021b; Fabijan *et al*., 2020).

The other set of KoRV variants present in the koala genome, known as recKoRVs, are recombinants between KoRV and an older retrotranspon, Phascolarctid endogenous retroelement (PhER) (Hobbs *et al*., 2017; Löber *et al*., 2018). This type of recombination between exogenous retroviruses and genomic transposons is well-described in other animal models such as cats and mice (Chiu & VandeWoude, 2021; Young *et al*., 2012) and can have considerable impact on the creation of viral variants with altered pathogenesis. These arise because of the way retroviruses replicate, involving jumps between two copies of viral RNA during the reverse transcription process, making them extremely prone to integrating other retroviral or even non-retroviral RNA into their genomes (Symer & Boeke, 2010). The recKoRVs were described as part of the koala reference genome analysis (Hobbs *et al*., 2017; Löber *et al*., 2018) and vary in copy number among animals, they are not functional as viruses and are unlikely to be able to retrotranspose themselves within the genome as they do not encode a complete reverse transcriptase reading frame. Our recent work has demonstrated that southern animals that test negative for KoRV with *pol* gene PCR or qPCR (and that would have previously been considered KoRV free) have recKoRV variants in their genomes (Tarlinton *et al*., 2021). These variants were found in all animals tested though do not appear to be fixed, with some loci (but not all) shared between animals from disparate genomic locations. They are not the same as those identified in northern animals, with the addition of an unidentified sequence between the KoRV *gag* and PhER reverse transcriptase sequence.

It is not clear what the significance of these recKoRV isolates are in koalas. They have most likely arisen and been transmitted alongside infectious KoRV variants as has been described for defective oncogene containing retroviruses in other species (Rubin, 2011). This implies that the ancestors of today's southern animals likely had infectious and or endogenous KoRV variants that were lost due to the extreme genetic bottleneck that the founder populations underwent during translocations. There is an additional possibility that the presence of these recKoRV variants may inhibit replication of infectious KoRV. Blockade of infectious virus has been described for endogenous retroviruses in other species including sheep and mice (Viginier *et al*., 2012; Nethe *et al*., 2005) and is thought to potentially be a driver of positive selection for endogenization of particular retroviral loci in genomes.

Elucidation of whether recKoRVs in southern animals have any effect on the lifecycle of infectious KoRV variants awaits further experimental work. This is not just an academic or evolutionary biology curiosity, in that these differences in KoRV prevalence and the linked prevalence of disease neatly distinguish the two largest genetic groups of animals in the range of the species. The recent bushfire events and translocations of animals associated with emergency responses and recovery have highlighted the fragility of the koala population in many areas. Consideration must be given to whether mixing of genetic populations should be avoided or whether this may have unintended consequences for either further population bottlenecks or infectious disease prevalence.

Acknowledgements. Funding was provided by the Queensland Department of the Environment and Heritage Koala Research Grant Programme the universities of Queensland and Nottingham, The VESKI Victoria Fellowship, and the Koala Life foundation. Many organizations and individuals provided access to clinical samples from koalas with especial thanks to Longleat Safari Park, Mogill Koala Rehabilitation Centre, the Adelaide Koala and Wildlife Hospital, and Fauna Rescue of South Australia.

References

Chiu, E. S., and S. VandeWoude. 2021. Endogenous Retroviruses Drive Resistance and Promotion of Exogenous Retroviral Homologs. *Annual Review of Animal Biosciences* 9: 225–248. https://doi.org/10.1146/annurev-animal-050620-101416

Fabijan, J., N. Sarker, N. Speight, H. Owen, J. Meers, G. Simmons, J. Seddon, R. D. Emes, R. Tarlinton, F. Hemmatzadeh, L. Woolford, and D. J. Trott. 2020. Pathological findings in retrovirus-positive koalas (*Phascolarctos cinereus*) from northern and southern Australia. *Journal of Comparative Pathology* 176: 50–66. https://doi.org/10.1016/j.jcpa.2020.02.003

Hobbs, M., A. King, R. Salinas, Z. Chen, K. Tsangaras, A. D. Greenwood, R. N. Johnson, K. Belov, M. R. Wilkins, and P. Timms. 2017. Long-read genome sequence assembly provides insight into ongoing retroviral invasion of the koala germline. *Scientific Reports* 7(1): 15838. https://doi.org/10.1038/s41598-017-16171-1

Johnson, R. N., D. O'Meally, Z. Chen, G. J. Etherington, S. Y. W. Ho, W. J. Nash, C. E. Grueber, *et al*. 2018. Adaptation and conservation insights from the koala genome. *Nature Genetics* 50: 1102–1111. https://doi.org/10.1038/s41588-018-0153-5

Joyce, B. A., M. D. Blyton, S. D. Johnston, P. R. Young, and K. J. Chappell. 2021. Koala retrovirus genetic diversity and transmission dynamics within captive koala populations. *Proceedings of the National Academy of Sciences, USA* 118: e2024021118. https://doi.org/10.1073/pnas.2024021118

Legione, A. R., J. L. S. Patterson, P. Whiteley, S. M. Firestone, M. Curnick, K. Bodley, M. Lynch, J. R. Gilkerson, F. M. Sansom, and J. M. Devlin. 2017. Koala retrovirus genotyping analyses reveal a low prevalence of KoRV-A in Victorian koalas and an association with clinical disease. *Journal of Medical Microbiology* 66(2): 236–244. https://doi.org/10.1099/jmm.0.000416

Löber, U., M. Hobbs, A. Dayaram, K. Tsangaras, K. Jones, D. E. Alquezar-Planas, Y. Ishida, J. Meers, J. Mayer, C. Quedenau, W. Chen, R. N. Johnson, P. Timms, P. R. Young, A. L. Roca, and A. D. Greenwood. 2018. Degradation and remobilization of endogenous retroviruses by recombination during the earliest stages of a germ-line invasion. *Proceedings of the National Academy of Sciences, USA* 115(34): 8609–8614. https://doi.org/10.1073/pnas.1807598115

McEwen, G. K., D. E. Alquezar-Planas, A. Dayaram, A. Gillett, R. Tarlinton, N. Mongan, K. J. Chappell, J. Henning, M. Tan, P. Timms, P. R. Young, A. L. Roca, and A. D. Greenwood. 2021. Retroviral integrations contribute to elevated host cancer rates during germline invasion. *Nature Communications* 12(1): 1316 https://doi.org/10.1038/s41467-021-21612-7

Neaves, L. E., *et al*. 2016. Phylogeography of the Koala, (*Phascolarctos cinereus*), and Harmonising Data to Inform Conservation. *PLoS One* 11(9): e0162207. https://doi.org/10.1371/journal.pone.0162207

Nethe, M., B. Berkhout, and A. C. van der Kuyl. 2005. Retroviral superinfection resistance. *Retrovirology* 2: 52. https://doi.org/10.1186/1742-4690-2-52

Quigley, B. L., V. A. Ong, J. Hanger, and P. Timms. 2018. Molecular dynamics and mode of transmission of koala retrovirus as it invades and spreads through a wild Queensland koala population. *Journal of Virology* 92(5): e01871-17. https://doi.org/10.1128/JVI.01871-17

Quigley, B. L., S. Phillips, O. Olagoke, A. Robbins, J. Hanger, and P. Timms. 2019. Changes in endogenous and exogenous koala retrovirus (KoRV) subtype expression over time reflects koala health outcomes. *Journal of Virology* 93(18): e00849-19. https://doi.org/10.1128/JVI.00849-19

Quigley, B. L., A. Melzer, W. Ellis, G. Tzipori, K. Nilsson, O. Olagoke, *et al*. 2021a. Koala retrovirus in northern Australia shows a mixture of stable endogenization and exogenous lineage diversification within fragmented koala populations. *Journal of Virology* 95: e02084-20. https://doi.org/10.1128/JVI.02084-20

Quigley, B. L., F. Wedrowicz, F. Hogan, and P. Timms. 2021b. Phylogenetic and geographical analysis of a retrovirus during the early stages of endogenous adaptation and exogenous spread in a new host. *Molecular Ecology* 30(11): 2626–2640. https://doi.org/10.1111/mec.15735

Robbin, A., J. Hanger, M. Jelocnick, B. L. Quigley, and P. Timms. 2020. Koala immunogenetics and chlamydial strain types are more directly involved in chlamydial disease progression in koalas from two south east Queensland koala populations than koala retrovirus subtypes. *Scientific Reports* 10(1): 15013. https://doi.org/10.1038/s41598-020-72050-2

Rubin, H. 2011. The early history of tumor virology: Rous, RIF, and RAV. *Proceedings of the National Academy of Sciences, USA* 108(35): 14389–14396. https://doi.org/10.1073/pnas.1108655108

Ruiz-Rodriguez, C. T., Y. Ishida, N. D. Murray, S. J. O'Brien, J. A. M. Graves, A. D. Greenwood, and A. L. Roca. 2016. Koalas (*Phascolarctos cinereus*) from Queensland are genetically distinct from 2 populations in Victoria. *Journal of Heredity* 107(7): 573–580. https://doi.org/10.1093/jhered/esw049

Sarker, N., J. Fabijan, J. Seddon, R. Tarlinton, H. Owen, G. Simmons, J. Thia, A. M. Blanchard, N. Speight, J. Kaler, R. D. Emes, L. Woolford, D. Trott, F. Hemmatzadeh, and J. Meers. 2019. Genetic diversity of koala retrovirus env gene subtypes: insights into northern and southern koala populations. *Journal of General Virology* 100(9): 1328–1339.
https://doi.org/10.1099/jgv.0.001304

Sarker, N., J. Fabijan, H. Owen, J. Seddon, G. Simmons, N. Speight, J. Kaler, L. Woolford, R. D. Emes, F. Hemmatzadeh, D. J. Trott, J. Meers, and R. E. Tarlinton. 2020. Koala retrovirus viral load and disease burden in distinct northern and southern koala populations. *Scientific Reports* 10(1): 263.
https://doi.org/10.1038/s41598-019-56546-0

Speight, N., B. Bacci, A. Stent, and P. Whiteley. 2020. Histological survey for oxalate nephrosis in Victorian koalas (*Phascolarctos cinereus*). *Australian Veterinary Journal* 98(9): 467–470.
https://doi.org/10.1111/avj.12986

Stephenson, T., N. Speight, W. Y. Low, L. Woolford, R. Tearle, and F. Hemmatzadeh. 2021. Molecular diagnosis of koala Retrovirus (KoRV) in South Australian koalas (*Phascolarctos cinereus*). *Animals* 11(5): 1477.
https://doi.org/10.3390/ani11051477

Symer, D. E., and J. D. Boeke. 2010. An everlasting war dance between retrotransposons and their metazoan hosts. In *Retroviruses*, ed. R. Kurth and N. Bannert. Norfolk: Caister Academic Press.

Tarlinton, R., J. Meers, J. Hanger, and P. R. Young. 2005. Real-time reverse transcriptase PCR for the endogenous koala retrovirus reveals an association between plasma viral load and neoplastic disease in koalas. *Journal of General Virology* 86(3): 783–787.
https://doi.org/10.1099/vir.0.80547-0

Tarlinton, R. E., N. Sarker, J. Fabijan, T. Dottorini, L. Woolford, J. Meers, G. Simmons, H. Owen, J. M. Seddon, F. Hemmatzadeh, D. Trott, N. Speight, and R. D. Emes. 2017. Differential and defective expression of Koala Retrovirus indicate complexity of host and virus evolution. *BioRxiv* 211466.
https://doi.org/10.1101/211466

Tarlinton, R. E., J. Meers, and P. R. Young. 2006. Retroviral invasion of the koala genome. *Nature* 442(7098): 79–81.
https://doi.org/10.1038/nature04841

Viginier, B., *et al*. 2012. Copy number variation and differential expression of a protective endogenous retrovirus in sheep. *PLoS One* 7(7): e41965.
https://doi.org/10.1371/journal.pone.0041965

Waugh, C. A., J. Hanger, J. Loader, A. King, M. Hobbs, R. Johnson, and P. Timms. 2017. Infection with koala retrovirus subgroup B (KoRV-B), but not KoRV-A, is associated with chlamydial disease in free-ranging koalas (*Phascolarctos cinereus*). *Scientific Reports* 7(1): 134.
https://doi.org/10.1038/s41598-017-00137-4

Xu, W., C. K. Stadler, K. Gorman, N. Jensen, D. Kim, H. Zheng, S. Tang, W. M. Switzer, G. W. Pye, and M. V. Eiden. 2013. An exogenous retrovirus isolated from koalas with malignant neoplasias in a US zoo. *Proceedings of the National Academy of Sciences, USA* 110(28): 11547–11552.
https://doi.org/10.1073/pnas.1304704110

Young, G.R., *et al*. 2012. Resurrection of endogenous retroviruses in antibody-deficient mice. *Nature* 491(7426): 774–778.
https://doi.org/10.1038/nature11599

Zheng, H., Y. Pan, S. Tang, G. W. Pye, C. K. Stadler, L. Vogelnest, K. V. Herrin, B. A. Rideout, and W. M. Switzer. 2020. Koala retrovirus diversity, transmissibility, and disease associations. *Retrovirology* 17: 34
https://doi.org/10.1186/s12977-020-00541-1