The Koala and its Retroviruses:
Implications for Sustainability and Survival

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The Origins and Ecological Impact of Koala Retrovirus

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Abstract. The genome of koala retrovirus (KoRV) has striking similarity to the gibbon ape leukemia virus (GALV) genome, suggesting the two viruses may share a common ancestor. Screening of DNA from a range of potential hosts of this putative ancestor virus revealed retroviral sequence from a grassland melomys *(Melomys burtoni)* that was closely related to sequence of both KoRV and GALV. This novel virus has been named *Melomys burtoni* retrovirus (MbRV). As grassland melomys and koalas share habitat, it is possible that there has been cross-species transmission of virus in the past.

Although a causative relationship between KoRV infection and disease in koalas is yet to be confirmed, koala populations with a high prevalence of KoRV infection have a higher incidence of diseases characteristic of retroviruses (cancer and immunosuppression) than populations with low KoRV-prevalence. Not all KoRV-infected koalas develop clinical disease. This variation in disease expression may result from differences in proviral (DNA) insertion sites among koalas, genetic variability of KoRV in different individuals or from variation in host genetics.

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Gammaretroviruses (RV) infect a large range of vertebrate hosts, and are causative agents of a number of diseases including lymphoid tumours and immunosuppression (Bendinelli *et al*., 1985; Rosenberg & Jolicoeur, 1997). Koala retrovirus (KoRV) is a relatively newly discovered retrovirus which is widespread throughout wild koala *(Phascolarctos cinereus)* populations in Australia (Hanger *et al*., 2000; Simmons *et al*., 2012). KoRV is of particular interest because it is the only known retrovirus currently undergoing a process of active endogenization in its host (Tarlinton *et al*., 2006). Koalas are known to suffer a high incidence of both chlamydiosis and cancer, and the high prevalence of KoRV has been suggested as a possible aetiological agent for immunosuppression and cancer in these animals (Tarlinton *et al*., 2008). Koala numbers in the wild have declined alarmingly since the beginning of European colonization and their geographic range has been significantly reduced. While the reasons for this decline are multi factorial, the high prevalence of KoRV and its apparent association with other diseases in koalas is a serious cause for concern.

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Origins of KoRV

Once the full sequence of KoRV was published, it was apparent that it shared striking genetic similarity with gibbon ape leukaemia virus (GALV), an exogenous, oncogenic retrovirus isolated from captive gibbons housed at the SEATO medical research facility in Bangkok, Thailand (Hanger *et al*., 2000). GALV and KoRV share such close identity that it seems likely they have a common ancestor. Since GALV was first isolated in the early 1970's there has been a degree of speculation about the source of this virus. Both GALV and KoRV are related to the murine leukaemia viruses and it has been suggested that a possible source of GALV is a related virus from a South East Asian rodent (Lieber *et al*., 1975; Callahan et *al*., 1979).

The link between KoRV and GALV adds further intrigue to this fascinating story given that a direct species jump between a primate and a marsupial that are geographically separated by several thousand kilometres seems unlikely. Following the screening of DNA from a number of potential vertebrate hosts, four partial proviral sequences from a novel retrovirus were obtained from a native Australian rodent, the grassland melomys *(Melomys burtoni)*. These sequences comprise a total of 2880 nucleotides and share remarkable identity with both KoRV and GALV. This virus has been named *Melomys burtoni* Retrovirus (MbRV) (Simmons, 2011). It shares such close identity with GALV that it could be considered another strain of GALV. Attempts to isolate infectious virus from the rodent host have so far been unsuccessful, although the provirus has open reading frames.

The grassland melomys and koala have overlapping geographic distributions throughout much of their range, and both are nocturnal (Redhead, 1983). This geographic overlap provides the opportunity for the two species to interact, and the close identity shared by KoRV and MbRV suggests there has been a cross species transmission of retrovirus between koalas and grassland melomys at some time in the past. Thus MbRV may well be the source of KoRV (or vice versa). However the genus *Melomys* does not occur in mainland South East Asia and so it seems unlikely MbRV is the direct source of GALV even though they share remarkable similarity. However *Melomys* species do occur in Papua New Guinea and it is possible MbRV may be part of a step wise transfer between several as yet unidentified species which led to the origin of the initial GALV outbreak. The discovery of GALV-related retroviral sequences in bats (Cui *et al.*, 2012) raises the possibility that these species may have been involved in this cross species transfer.

Ecological impact of KoRV infection

There is clear evidence that many retroviruses cause disease in their respective hosts. Examples include feline leukaemia virus, equine infectious anaemia, GALV and others. However the same is not true for KoRV at the present time. The link between KoRV infection and disease in koalas is at this stage more of an association rather than a demonstrated cause and effect, and although there are alarming associations between KoRV prevalence and disease in koalas more research is needed in order to clarify the role of KoRV as a pathogen. A discussion on the ecological impact of KoRV therefore needs to be addressed with this in mind.

What is clear is that wild koala populations in Australia appear to have different disease spectra depending on their KoRV status and location. Populations in the north, particularly in Queensland, but also further south, have well-documented high levels of disease whilst some southern populations, for example Kangaroo Island, are virtually free of chlamydiosis and cancer (Ladds, 2009; G. Johnsson, pers. comm. 2008). The reasons for these differences do not simply appear to be the presence or absence of KoRV and are likely more complex. Some of the possible mechanisms by which KoRV may cause disease are discussed below.

Endogenous versus exogenous

One variable which may affect the impact of KoRV is the fact that it appears to currently be in the active process of endogenization, at least in the north (Tarlinton *et al.,* 2006). When proviral copy numbers were compared between KoRV positive koalas from Queensland and Victoria the results were strikingly different. In Queensland, average proviral copy number/cell nucleus was high (165) and surprisingly uniform between animals. For Victorian koalas the number varied from about one/cell to as low as 1/10,000 cells (Simmons *et al*., 2012). Thus while KoRV appears to be endogenous in Queensland, in at least some Victorian koalas it may be present only in its exogenous form. While proviral copy number is fairly uniform in Queensland koalas, the loci where the insertions occur are variable (Tarlinton *et al.*, 2006). This may in part explain why some koalas are able to live into old age without clinical signs of disease while others succumb to chlamydiosis or cancer when relatively young. For example some koalas may have insertions in regions of their genome that impact on gene expression to the detriment of the individual's long term survivability, whereas in other koalas the proviral loci may occur in less critical regions.

In populations where the virus may be present in its exogenous form the spectrum of disease appears to be less severe. Indeed on Kangaroo Island, where the population was introduced in the 1920's and which is now highly inbred, koalas are thriving to such an extent that they are destroying their habitat.

Genetic variation

Genetic variability in both koalas and KoRV may help explain the differences in disease expression seen in different koala populations. Current research is demonstrating genetic differences in strains of KoRV isolated from different koalas and apparent differences in pathogenicity of these different strains. In addition there may well be genetic differences between koala populations which affect susceptibility to disease. For example a study of the mitochondrial control region in koalas from different populations demonstrated significant differentiation in mtDNA haplotype frequencies in these different groups (Houlden *et al*., 1999). The possible influence of such genetic variability in koala populations on the pathogenicity of KoRV remains to be investigated.

Oncogenesis

Koala retrovirus does not appear to be as oncogenic as its near relative GALV, which rapidly causes leukaemia in infected gibbons (Kawakami *et al.*, 1980). While there is an alarming incidence of cancer in koalas, it is also true that the many KoRV positive animals remain healthy. Areas with some of the highest prevalence of neoplastic disease appear to be in the north, while on Kangaroo Island cancer and chlamydiosis are rarely if ever seen (Johnnson, 2008). Whether this apparent lower incidence of disease on Kangaroo Island is due to the lower prevalence of KoRV in this population or other factors related to differences in virulence and/or genetic susceptibility of koalas remains unknown.

KoRV viraemia and disease

A study in 2005 investigated levels of KoRV viraemia and incidence of disease in 90 captive and free ranging koalas. There was a significantly higher incidence of cancer in the high viraemic group, although no significant differences between levels of viraemia and severity of chlamydiosis could be demonstrated (Tarlinton, 2005). In a later study which involved 100 wild koalas from south east Queensland, 40 animals had clinical signs of chlamydiosis and one had cancer. This study failed to demonstrate a significant link between the level of viraemia and chlamydiosis or other disease (Simmons, 2011).

Conclusions

While there are a number of serious threats to the koala's long-term survival, including habitat destruction and urban expansion, the widespread prevalence of KoRV and its association with disease in koalas is also a cause for concern. There are still large gaps in our knowledge about the pathogenesis of KoRV and variability in disease status seen in different koala populations and there is a real need for further research in this area. A potential step that could be taken to mitigate the possible negative impact of KoRV on koala viability in the shorter term could include establishment of KoRV free populations in areas of suitable habitat. Furthermore, breeding programs in the north that used KoRV animals with a family history of lack of disease susceptibility might also limit detrimental outcomes and increase survivability of these animals.

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