The Koala and its Retroviruses:
Implications for Sustainability and Survival

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The Evolution of Koala Retroviruses: Insights from other Endogenous Retroviruses

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Abstract. The koala retrovirus (KoRV) is associated with outbreaks of *Chlamydia* and leukemia in wild and zoo koalas *(Phascolarctos cinereus)*. Although endogenous retrovirus-like elements (ERVs) are common in the genomes of all vertebrates (comprising ca 8% of the human genome), KoRV is the only retrovirus known to be currently in the process of transitioning from exogenous to endogenous form. Here, we examine how other host-pathogen interactions, including other host-ERV systems, can inform our understanding of KoRV in koalas. We note that as an exogenous retrovirus becomes endogenous, there would be a dramatic reduction in mutation rates, which may shift the process of accommodation from the pathogen to the host. The low genetic diversity present in koalas may be in part responsible for the failure of the species to develop genetic resistance to KoRV. Isolation between koala populations may have hindered the geographic spread of the virus, but may also hinder selective sweeps of beneficial host alleles or beneficial proviral mutations, thereby precluding rapid increases in host fitness. In humans, some ERVs are involved in normal host functions such as placentation, or in the pathogenesis of diseases such as Hodgkin's lymphoma. However, ERVs present in humans and other species are ancient, precluding prospective studies of germ line invasions. By contrast, the ongoing invasion of the koala germ line by KoRV provides a singular opportunity to study retroviral endogenization as it is occurring. This research can benefit the health of both humans and koalas.

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Endogenous retroviruses are common elements present in the genomes of all vertebrates examined, with ca. 8% of the human genome comprised of retrovirus-like elements (Bromham, 2002; Weiss, 2006; Pontius *et al.*, 2007; Blikstad *et al.*, 2008). Although some ERVs play a functional role in host health and disease in humans and other species (Roy-Burman, 1995; Mi *et al.*, 2000; Lamprecht *et al.*, 2010), most ERVs exist as "junk DNA" with highly disrupted coding regions and no functional role (Roca *et al.*, 2004; Roca *et al.*, 2005; Pontius *et al.*, 2007). Comparisons across the genomes of humans and other primates, and of other vertebrate lineages, have shown that ERVs have resulted from multiple invasions of and proliferations in the host germ line by retroviruses (Johnson & Coffin, 1999; Blikstad *et al.*, 2008; Polani *et al.*, 2010). Despite being ubiquitous, almost all known ERVs endogenized many thousands or millions of generations ago, making it difficult to infer the events that occur during and shortly after the invasion of a host germ line by an endogenizing retrovirus (Weiss, 2006; Blikstad *et al.*, 2008).

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The koala retrovirus (KoRV) appears to represent an exceptionally recent invasion of a host germ line by a retrovirus (Hanger *et al.*, 2000; Stoye, 2006; Tarlinton *et al.*, 2006). Unlike any other known ERV, KoRV appears to be present in endogenous form in only some but not all members of the host species (Stoye, 2006; Tarlinton *et al.*, 2006; Simmons *et al.*, 2012). Some populations of koala in southern Australia appear to be free or largely free of KoRV (Stoye, 2006; Tarlinton *et al.*; 2006; Simmons *et al.*, 2012). KoRV also appears to persist as an exogenous virus, and thus provides the opportunity to study the transition of a retrovirus from exogenous to endogenous form on a "real time" basis (Stoye, 2006; Tarlinton *et al.*, 2006; Simmons *et al.*, 2012).

KoRV is associated with pathologies that affect both wild and zoo koalas, most notably *Chlamydia* infection and the formation of leukemias (Canfield *et al.*, 1988; Hanger *et al.*, 2000; Tarlinton *et al.*, 2005; Fiebig *et al.*, 2006; Oliveira *et al.*, 2006; Oliveira *et al.*, 2007). In recent studies, we found that the functional features present today in KoRV have remained largely unchanged for more than a century (Ávila-Arcos *et al.*, 2013). We also found that KoRV was already ubiquitous in northern Australian populations in the late 1800s, suggesting that the spread of KoRV geographically has been limited since at least that time (Ávila-Arcos *et al.*, 2013). Finally, the genetic variability of koalas, previously reported to be low in living populations, was found to be similarly low in ancient museum samples as well (Tsangaras *et al.*, 2012). Here we examine how other host-pathogen systems can inform approaches to KoRV in koalas. We specifically examine other host-ERV interactions and how they can inform our understanding of KoRV, although the examples will include non-ERV and non-retroviral examples when these appear to be relevant.

Host-pathogen accommodation: potential role for population size and mutation rates

The evolution of a host-pathogen system may involve a process of co-adaptation between the pathogen and the host (Kerr, 2012). When a pathogen enters a new species, it may be especially pathogenic to the novel host. However, there may in some cases be evolutionary pressures for a virus to become less pathogenic over time (Kerr, 2012). Host genetic variation that provides resistance to the virus will be selected for, and any host variant that provides protection would be expected to undergo a selective sweep, becoming more common in the population (May & Anderson, 1979).

An important model for host-pathogen interaction is the myxoma virus infection of European rabbits in Australia (Kerr, 2012). Myxoma virus is a poxvirus naturally found in and benign to American rabbits (genus *Sylvilagus*). However, the virus is deadly to European rabbits, which are an invasive species and a major pest in Australia. In 1950, myxoma virus was released into the Australian rabbit population, spreading quickly across the continent. Initially the case fatality rate for infected rabbits was 99.8% (Kerr, 2012). But the virus quickly became attenuated, with a case fatality rate of 90% by the second season, suggesting that there was selective pressure, if only initially (May & Anderson, 1990), for the virus to become less deadly (Kerr, 2012). In time, the host species also became more resistant to the virus. Rabbits exposed to one particular grade of virus went from 90% to 26% fatality over 7 generations, as genetic variants that made rabbits less susceptible to the virus became more common each generation (Kerr, 2012).

In considering the adaptation of myxoma virus and rabbits to each other, it is important to note that adaptations are likely to impact the pathogen population more quickly than they impact the host population (Mulvey *et al.*, 1991; Kerr, 2012). The genetic variation present within a lineage varies with mutation rate and population size (Tajima *et al.*, 1998; Duffy *et al.*, 2008). Each infected rabbit may carry a very large number of copies of the virus, thus the population size of the virus would be greater than that of affected rabbits, and the virus would also have a shorter generation time (Mulvey *et al.*, 1991; Duffy *et al.*, 2008; Kerr, 2012). This in turn would lead to a relatively larger number of new mutations in the virus, which would allow for greater adaptability of the virus to the rabbit than vice versa (Mulvey *et al.*, 1991; Duffy *et al.*, 2008).

This example of host-pathogen co-adaptation may be relevant to the koala-KoRV system. When KoRV first infected koalas as an exogenous retrovirus, the virus rather than the koala may have undergone most of the initial mutation that would drive the host and parasite to accommodate each other (Duffy *et al.*, 2008). This may be especially true given that koalas appear to suffer from reduced genetic diversity (Wilmer *et al.*, 1993; Tsangaras *et al.*, 2012). KoRV appears to have developed a number of protein motifs that reduce its virulence vs. the closely related gibbon ape leukemia virus (GALV) (Oliveira *et al.*, 2006; Oliveira *et al.*, 2007). It may not be surprising that KoRV appears to have evolved this lowered virulence before becoming endogenized (Ávila-Arcos *et al.*, 2013). Invasion of the koala germ line by KoRV may have been difficult before the mitigating mutations, since any endogenous KoRV that killed its host before it reached reproductive age could not have persisted. A greater understanding of why KoRV is currently not deadly enough to prevent sufficient numbers of host offspring from reaching reproductive age may provide insights into how to also protect older koalas.

KoRV would be present in very high copy number in each infected koala, thus having a much higher population size than the koala host (Duffy *et al.*, 2008). Furthermore, exogenous KoRV, with an RNA genome that lacks the genomic repair mechanisms of the host, would have a much higher mutation rate than the koala, which has DNA repair mechanisms that limit the mutation rate (Duffy *et al.*, 2008).

One of the critical recent findings made by our group is that KoRV has changed little in the past century (Ávila-Arcos *et al.*, 2013). This may be due to the reduction in mutation rate that would occur once a retrovirus endogenizes (Duffy *et al.*, 2008). Once endogenized, KoRV becomes subject to cellular DNA-repair mechanisms. Thus the mutation rate for endogenous KoRV is likely to be substantially lower than the rate for exogenous KoRV, slowing the adaptive potential of the retrovirus relative to that of the host, once the virus transitions to endogenous copies.

Adaptation between ERV and host: the evolution of protective ERVs

KoRV is the only ERV for which some individuals of the host species are believed to be completely free of proviral copies (Stoye, 2006; Tarlinton *et al.*, 2006; Tarlinton *et al.*, 2008; Simmons *et al.*, 2012). In other host species ERVs may be insertionally polymorphic, i.e., present at a particular locus in only some individuals (Turner *et al.*, 2001; Roca *et al.*, 2005). Nonetheless, even in these cases, all members of the species will carry ERV copies at other loci (Turner *et al.*, 2001; Roca *et al.*, 2005). In the case of KoRV, many individuals especially in southern populations may be completely free of endogenous proviruses, an indication that the germ line of the koala has only been invaded recently relative to known

ERVs in other species (Stoye, 2006; Tarlinton *et al.*, 2006; Tarlinton *et al.*, 2008; Simmons *et al.*, 2012). Furthermore, KoRV appears to be strongly pathogenic in koalas (Hanger *et al.*, 2000; Tarlinton *et al.*, 2005; Oliveira *et al.*, 2006; Oliveira *et al.*, 2007; Tarlinton *et al.*, 2008), while most ERVs in other species appear to be benign. Since vertical transmission in general tends to select for lower virulence (Toft & Karter, 1990), this may be another indication of a recent origin for KoRV. An examination of how ERVs in other species may have become innocuous may provide insights into the future of KoRV in the koala.

One relevant example may be the endogenous feline leukemia viruses (enFeLVs) present in the germ line of the domestic cat and related species (Polani *et al.*, 2010). The presence of enFeLVs in several closely related species of the genus *Felis* suggests that these ERVs began proliferating in the germ line of an ancestor of domestic and wild cats some 3–6 million years ago (Johnson *et al.*, 2006; Polani *et al.*, 2010). That has been sufficient time for many enFeLVs to develop mutations that disrupt the open reading frames (ORFs) of the provirus, although at least one copy of enFeLV retains its ORF structure, indicative of a relatively recent integration event (Roca *et al.*, 2004; Pontius *et al.*, 2007). Mutations in enFeLV after it endogenized would occur at the slow rate of change that occurs in the genome of the host species (Roca *et al.*, 2004). Yet even this slow rate has been sufficient to disrupt most copies of enFeLV in the domestic cat, rendering enFeLVs non-functional due to frame-shift or other disruptive mutations, or to other mechanisms that can block the proliferation of selfish DNA (Roca *et al.*, 2004; Pontius *et al.*, 2007). The high pathogenicity of KoRV in koalas may suggest that insufficient time has elapsed for a general breakdown of the structure of genomic copies of KoRV, although further studies would be needed to establish this definitively.

Interestingly, some enFeLVs in the cat germ line appear to play a protective role in the host species. It appears that viral transcripts of the *env* gene encoded by a domestic cat enFeLV produce partial envelope protein, which is secreted by cells (McDougall *et al.*, 1994). This partial protein appears to block entry into the cells of exogenous FeLV of strains that share envelope similarity with the endogenous forms (McDougall *et al.*, 1994). Thus, an enFeLV codes for an envelope protein that interferes with infection by similar exogenous viruses (McDougall *et al.*, 1994). Such a protective effect would be expected to lead to positive selection, increasing the frequencies of the protective ERV in host populations. An analogous protective role also appears to have evolved in some mice within the genus *Mus*. In mice, a retroviral restriction gene *Fv1*, has been found to be derived from the *gag* region of an ERV (Best *et al.*, 1996; Yan *et al.*, 2009). This ERV appears to code for a protein product that appears to interact with exogenous murine leukemia viruses, restricting the ability of the exogenous virus to proliferate (Best *et al.*, 1996; Yan *et al.*, 2009).

Koala biology and protective host genetic variants against KoRV

While ERVs may develop a protective role within the host, there is also evidence that some host genetic variants will provide protection against retroviruses. Protective allelic variants in the host species would be expected to increase over time due to selective pressure by the pathogen against individuals that lack protection (May & Anderson, 1979). Host genes with allelic variants that mediate responses to retroviruses have been well studied in the case of human immunodeficiency virus (HIV-1) (O'Brien & Nelson, 2004; An & Winkler, 2010; Zhao *et al.*, 2012). Several dozen human genes have been found to have allelic variants that are beneficial (or detrimental) to humans exposed to HIV-1 (O'Brien & Nelson, 2004; An & Winkler, 2010; Zhao *et al.*, 2012). For example, HIV-1 uses the transmembrane receptor CCR5 to enter and infect host cells (Lederman *et al.*, 2006). About 10% of humans of north European ancestry carry a variant called *CCR5*-delta32, in which the gene is disrupted by a deletion (Liu *et al.*, 1996; Lederman *et al.*, 2006). Individuals with one or two copies of the mutant allele are much less susceptible to HIV-1 infection than wild type individuals (Liu *et al.*, 1996; Lederman *et al.*, 2006). In humans, host genes with allelic variants protective against HIV-1 fall into several categories, and may represent HIV co-receptors, immune modifiers (HLA and cytokines) or post-entry retroviral restriction factors (An & Winkler, 2010; Zhao *et al.*, 2012).

No protective variants against KoRV have yet been identified in the koala. Nonetheless, one may consider whether genes with analogous function in the koala currently have (or may develop through mutation) allelic variants that would be protective against KoRV. One may also consider whether some endogenous copies of KoRV may eventually develop a protective role against exogenous KoRV. In either case, aspects of koala biology may be relevant to the development of resistance against KoRV, whether potentially mediated by a protective endogenous KoRV, or by host genetic variants resistant against the virus. Koalas appear to have a low degree of genetic variation, and this low variation appears to have been present in the species for more than a century (Wilmer *et al.*, 1993; Tsangaras *et al.*, 2012). The lack of host genetic variants may limit the diversity of potential retroviral restriction genes, and thus limit the ability of resistance against KoRV to increase over time in the population (May & Anderson, 1979).

Another factor that may affect host-retroviral interactions is limited dispersal or fragmented range of the host (May & Anderson, 1990). The high geographic segregation of mtDNA haplotypes suggests that female koalas may have experienced limited dispersal or that gene flow may have been limited by the fragmentation of species range (Wilmer *et al.*, 1993; Taylor *et al.*, 1997; Houlden *et al.*, 1999; Fowler *et al.*, 2000; Tsangaras *et al.*, 2012; Ávila-Arcos *et al.*, 2013). Isolation of koala populations may have been beneficial in potentially slowing the spread of KoRV from north to south. However, such isolation could also have a strongly negative consequence: in order for a selective sweep to occur, there must be geographic dispersal of the genetic variants that confer fitness (Petit & Excoffier, 2009). Limited dispersal or isolation of populations would limit the degree to which selective sweeps of fitness-promoting variants could occur. Protective effects, whether mediated by endogenous KoRVs that developed a protective role, or mediated by beneficial host genetic variants, could not undergo beneficial selective sweeps in a host population that has limited gene flow (Petit

& Excoffier, 2009). One may even speculate that locally protective variants could potentially be evolving in the koala population separately, but with an inability to improve fitness across the species due to limited geographic dispersal or connectivity (Tack *et al.*, 2012).

KoRV and biomedical research: towards an understanding of koala and human ERVs

Human endogenous retroviruses (HERVs) and related elements comprise ca. 8% of the human genome, a larger proportion than is accounted for by protein-coding genes (Jern & Coffin, 2008). Most HERVs are considered to be non-functional "junk" DNA (Jern & Coffin, 2008). However, recently several HERVs have been established to play a role in human health and disease. For example, the gene *syncytin* plays a functional role in human placental formation (Mi *et al.*, 2000). *Syncytin* is derived from a HERV that entered the germ line of a primate ancestor of the human lineage, since the gene is also present in apes and old world monkeys. The syncytin protein plays a role in formation of the syncytiotrophoblast, a multi-nucleated structure that is vital for normal placentation. Thus, an ERV has been co-opted by its host lineage to play a critical function in the host organism. Interestingly, analogous use of endogenous retroviruses has now been found in rodents, sheep, and other species (Cornelis *et al.*, 2013). Yet the ERVs that play a role in placentation do not derive from a common ancestral invasion of the germ line by the same ERV. Rather, it appears that different ERVs that invaded the germ lines of different mammalian ancestors have been co-opted for placentation across different lineages (Cornelis *et al.*, 2013).

Detrimental long-term effects have also been established for ERVs in various species. Although a role for HERVs has been proposed for many diseases (Voisset *et al.*, 2008), only recently has a direct role in a human disease been established. In Hodgkin's lymphoma in humans, one of the critical steps leading to formation of the disease involves de-repression of an ERV promoter (Lamprecht *et al.*, 2010). Activation of this promoter plays a central role in tumor cell survival (Lamprecht *et al.*, 2010). One reason that it may be difficult to establish a role for ERVs in other diseases is that the human complement of ERVs will be quite different from the ERVs present in biomedical model organisms such as the mouse. The mouse lineage is separated from the human lineage by 85 million years of evolution, involving completely independent invasions of the germ line by ERVs during that time (Johnson & Coffin, 1999; Murphy *et al.*, 2001). Thus diseases caused by ERVs in commonly studied biomedical model organisms may be quite different from those caused by HERVs in humans, and vice versa.

Given that organisms commonly relied upon for biomedical studies may not be directly suitable models for human ERVs, and given that most ERVs, including HERVs, invaded their host germ lines thousands or millions of generations ago, the ongoing invasion of the koala germ line by KoRV may be of great biomedical importance (Hanger *et al.*, 2000; Tarlinton *et al.*, 2005; Fiebig *et al.*, 2006; Oliveira *et al.*, 2006; Stoye, 2006; Tarlinton *et al.*, 2006; Denner, 2007; Tarlinton *et al.*, 2008; Langhammer *et al.*, 2011; Miyazawa *et al.*, 2011; Cui *et al.*, 2012; Denner, 2012; Simmons *et al.*, 2012; Ávila-Arcos *et al.*, 2013; Shojima *et al.*, 2013). The transitioning of KoRV from exogenous retrovirus to endogenous provirus is currently underway, and this represents an excellent, and so far the only, opportunity for studying the process of retroviral germ line invasion prospectively rather than retrospectively (Stoye, 2006; Tarlinton *et al.*, 2006; Tarlinton *et al.*, 2008). This potential utility of koalas and KoRV for understanding the origins of 8% of the human genome should also be seen as potentially beneficial to the koala (Fiebig *et al.*, 2006). Even if some biomedical studies of KoRV have as their primary goal insights into the processes that gave rise to ERVs in humans, any information gained from biomedical studies that increase our understanding of KoRV will necessarily increase our ability to help koalas afflicted with the virus.

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