Contrasting patterns of coding and flanking region evolution in mammalian keratin associated protein-1 genes

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Abstract

DNA repeats are common elements in eukaryotic genomes, and their multi-copy nature provides the opportunity for genetic exchange. This exchange can produce altered evolutionary patterns, including concerted evolution where within genome repeat copies are more similar to each other than to orthologous repeats in related species. Here we investigated the genetic architecture of the keratin-associated protein (KAP) gene family, *KRTAP1*. This family encodes proteins that are important components of hair and wool in mammals, and the genes are present in tandem copies. Comparison of *KRTAP1* gene repeats from species across the mammalian phylogeny shows strongly contrasting evolutionary patterns between the coding regions, which have a concerted evolution pattern, and the flanking regions, which have a normal, radiating pattern of evolution. This dichotomy in evolutionary pattern transitions abruptly at the start and stop codons, and we show it is not the result of purifying selection acting to maintain species-specific protein sequences, nor of codon adaptation or reverse transcription of *KRTAP1-n* mRNA. Instead, the results are consistent with short-tract gene conversion events coupled with selection for these events in the coding region driving the contrasting evolutionary patterns found in the *KRTAP1* repeats. Our work shows the power that repeat recombination has to complement selection and finely tune the sequences of repetitive genes. Interplay between selection and recombination may be a more common mechanism than currently appreciated for achieving specific adaptive outcomes in the many eukaryotic multi-gene families, and our work argues for greater emphasis on exploring the sequence structures of these families.

54 **INTRODUCTION**

55 Repetitive DNA is widespread in most eukaryote genomes (BRITTEN AND KOHNE 1968;
56 RICHARD *et al.* 2008; LOPEZ-FLORES AND GARRIDO-RAMOS 2012). There are two basic repeat
57 DNA types: tandem repeats that are typically arranged in head-to-tail arrays; and dispersed
58 repeats, and these can occur in either coding or non-coding DNA. Repeats are thought to
59 arise from recombination-based duplication/amplification events (STEPHAN 1989). Sequence
60 identity between duplicates will then decay through the diversifying force of mutation, unless
61 counteracting processes operate (BROWN *et al.* 1972; DOVER 1982). The balance between
62 duplication, diversification, selection, and counteracting forces thus dictate the evolutionary
63 dynamics of repeats. Two main paradigms have been proposed to account for the long-term
64 maintenance of repeat identity: concerted evolution and birth-and-death evolution. Concerted
65 evolution describes a pattern of evolution where the repeats within a genome show greater
66 sequence identity to each other than to orthologous repeats in related genomes (ELDER AND
67 TURNER 1995). The pattern of concerted evolution is proposed to result from recombination-
68 based processes, such as gene conversion and unequal cross-over events, that replace the
69 DNA sequence from one repeat with that from another repeat (LIAO 1999). In so doing, these
70 recombination processes maintain sequence identity between repeat copies in the face of
71 mutation, and thus homogenize the repeats (DOVER 1982). 'Birth-and-death' evolution
72 involves purifying selection maintaining sequence identity between repeats that are generated
73 by occasional duplication events (i.e. birth), as well as death, which results from repeat loss
74 or pseudogenization (NEI *et al.* 1997; NEI *et al.* 2000). While there has been debate as to
75 which of these processes best describes the evolutionary dynamics of repetitive DNA (NEI
76 AND ROONEY 2005; ROONEY AND WARD 2005; EIRIN-LOPEZ *et al.* 2012), a basic
77 characterization of the evolutionary dynamics of most repeat families is lacking.

78 The keratin-associated proteins (KAPs) are a diverse group of proteins, and are rich in either
79 sulphur, or glycine and tyrosine. They are important structural components of hair and wool
80 fibres, and form a matrix that cross-links the keratin intermediate filaments. The genes
81 encoding the KAPs are called *KRTAP*s (GONG *et al.* 2012), and can be classified into 27
82 families, with each family comprising 1-12 members that are usually tandemly arranged
83 (ROGERS AND SCHWEIZER 2005; ROGERS *et al.* 2006; GONG *et al.* 2016). The *KRTAP*s are
84 single exon (intron-less) genes, with small coding sequences (less than 1 kb) (ROGERS AND
85 SCHWEIZER 2005), and they have low numbers of pseudogenes. For example, in humans the
86 pseudogene:gene *KRTAP* ratio is approximately 1:5 (GONG *et al.* 2016), while across all
87 human genes the ratio is close to 1:1 (TORRENTS *et al.* 2003; STEIN 2004). In addition, the
88 *KRTAP*s show high levels of population variation, with all known *KRTAP* genes being
89 polymorphic in sheep (GONG *et al.* 2010b; GONG *et al.* 2016; ZHOU *et al.* 2016), where they
90 are well studied because of their roles in determining wool phenotypes (ZHOU *et al.* 2015; LI
91 *et al.* 2017a; LI *et al.* 2017b; LI *et al.* 2017c; TAO *et al.* 2017a; TAO *et al.* 2017b). Despite this
92 variation, it has been reported that at least some *KRTAP* genes show a pattern of concerted
93 evolution between the paraglogous gene copies (ROGERS *et al.* 1994; WU *et al.* 2008; KHAN
94 *et al.* 2014).

95 The KAP1 proteins form the best characterised KAP family, and they show a high degree of
96 sequence heterogeneity compared to other KAP families. These KAP1 proteins appear to be
97 restricted in expression to the middle to upper cortex region of the hair and wool follicle, and
98 are absent in the cuticle (POWELL AND ROGERS 1997; SHIMOMURA *et al.* 2002). Their precise
99 role in hair and wool function, has yet to be determined. The genes encoding the KAP1
100 proteins (*KRTAP1-n*) have been characterized in a number of mammalian species, where they
101 are usually arranged as four tandem copies (**Figure 1**) (KHAN *et al.* 2014). The coding
102 regions of the *KRTAP1-n* genes vary in length within species, predominantly as a

103 consequence of variation in the number of imperfect tandem decapeptide repeat units (GONG
104 *et al.* 2016) (**Figure 1**).

105 Here we analyse the *KRTAP1* genes from a number of mammalian species, including four
106 species for which the *KRTAP1-n* loci have not been described. Together with the existing
107 *KRTAP1-n* sequences, we reveal that the *KRTAP1-n* coding regions display a pattern of
108 concerted evolution. In stark contrast to the coding region though, we find that the repeat
109 flanking regions display no evidence of concerted evolution, and instead appear to be
110 evolving by normal vertical or radiating evolution. Surprisingly, we find that this pattern of
111 coding region restricted concerted evolution is not the result of purifying selection, nor does
112 it result from codon adaptation or reverse transcription/reintegration of *KRTAP1-n* mRNA
113 sequences. Instead, the results are best explained by a combination of on-going short-tract
114 gene conversion events between the *KRTAP1-n* copies, and negative selection. We argue that
115 these gene conversion events act as an unusual mechanism of purifying selection to prevent
116 excessive intra-genomic divergence between the four gene copies, while also allowing inter-
117 species diversity. This unusual mode of evolution may apply to other multicopy genes that
118 encode products subject to diversifying selection.

119

120

121 **MATERIALS AND METHODS**

122 **Sequence Resources and Gene Identification:** All genome sequences were sourced from
123 the NCBI GenBank. Previously identified *KRTAP1-n* sequences (ITENGE-MWEZA *et al.* 2007;
124 WU *et al.* 2008; GONG *et al.* 2010a; GONG *et al.* 2011) were used to search the genomes of
125 cattle, horses, rabbits and African elephants using BLAST with default parameters, and the

genes retrieved were identified by sequence identity within both the coding and flanking regions (**Table S1**).

Sequence Alignments: *KRTAP1* nucleotide sequences (**Table S1)** for all four paralogs from the ten species (sheep, cattle, dog, elephant, horse, human, macaque, mouse, rat and rabbit) were separated into 5' flanking regions, coding sequences, and 3' flanking regions. The multiple sequence alignment tool *mafft* (v7.123b) (KATOH AND STANDLEY 2013) was used to separately align the 5' and 3' flanking regions as nucleotide sequences, using the arguments '--nuc --localpair --maxiterate 1000'. To align the coding sequences at the predicted amino acid level, *mafft* with the arguments '--amino --localpair --maxiterate 1000' was run.

The coding sequence alignment was subsequently reverse translated using *revTrans* (v1.4) (WERNERSSON AND PEDERSEN 2003) with two input files: the sequences of all the coding regions, and the amino acid sequence alignments. The sequences in the two files were paired by name using the '-match name' parameter, and default values were used for all other parameters. A number of regions align poorly and have many indels, therefore we used the longest continuous coding sequence block (198 nucleotides; covers on average around 40% of the coding region) where none of the 40 sequences had indels. For the flanking region alignments, we used *Gblocks* (v0.91b) (TALAVERA AND CASTRESANA 2007) to select blocks that cover approximately 40% of the flanking regions having the best alignment. We also used *Gblocks* with less stringent criteria to create multiple sequence alignments of the coding and flanking regions that included more poorly aligning regions.

Phylogenetic Trees: *PhyML* (v3.1) (GUINDON *et al.* 2010) was used to construct phylogenies based on the coding and flanking region sequences. The number of resampled bootstrap data sets was set to 1000 (parameter '-b 1000'), and the additional arguments '-q -s BEST -o tlr'

149 were employed. The Bioconductor package *ggtree* (v1.9.4) (YU *et al.* 2017) was used to plot
150 the phylogenies.

151 **Codon Adaptation Index:** The CAIcal server (http://genomes.urv.es/CAIcal(PUIGBO *et al.*
152 2008) was used to calculate CAI values for the *KRTAP1*s, as well as expected CAI values
153 from permutated sequences using default parameters and published codon usage data
154 (NAKAMURA *et al.* 2000).

155 **Motifs in the Coding Sequences:** We used MEME motif finder (v4.12.0) (BAILEY *et al.*
156 2006) to explore repetitive elements in the coding sequences. The repetitive structure of the
157 coding regions reported in the Results was obtained with parameters '-dna -oc . -nostatus -
158 time 18000 -maxsize 60000 -mod anr -nmotifs 6 -minw 6 -maxw 30 -minsites 20 -maxsites
159 600 –revcomp' and all the other parameters set to the default values.

160 ***KRTAP1-n* Polymorphism in Sheep:** Intra-specific variation was assessed using three
161 sequences for *KRTAP1-1* (ITENGE-MWEZA *et al.* 2007), eleven sequences for *KRTAP1-2*
162 (GONG *et al.* 2011; GONG *et al.* 2015), nine sequences for *KRTAP1-3* (ITENGE-MWEZA *et al.*
163 2007), and nine sequences for *KRTAP1-4* (GONG *et al.* 2010a). These were aligned using
164 DNAMAN (v5.2.10; Lynnon BioSoft, Canada) with default parameters, and polymorphic
165 sites were identified manually.

166 **Data Availability Statement:** Sequence data are available at GenBank and the accession
167 numbers and positions are listed in the **Materials and Methods** (sheep polymorphism data)
168 and Table S1 (*KRTAP1* sequences). Descriptions of the supplemental material are given in
169 **File S1,** and the supplemental material are available on figshare.

170

171

172 **RESULTS**

173 **Mammalian *KRTAP1-n* repeats show a concerted evolution pattern in the coding but**
174 **not the flanking regions**

175 To better understand the genetic architecture of the mammalian *KRTAP1* cluster, we selected
176 the *KRTAP1* genomic region from key members of the mammalian phylogeny for analysis.
177 The Basic Local Alignment Search Tool (BLAST) was used to search GenBank with known
178 *KRTAP1-n* sequences to identify and retrieve the *KRTAP1* clusters from the genomes of four
179 species (cattle, horses, rabbits and African elephants) for whom *KRTAP1-n* sequence
180 information has not been reported (**Figure S1**). We then combined these with previously-
181 identified *KRTAP1-n* sequences from other mammalian species to obtain sampling across the
182 mammalian phylogeny (**Figure 2**).

183 Previously, the *KRTAP1* genes of sheep were shown to contain a variable number of
184 occurrences of a QTSCCQPXXX decapeptide tandem repeat in the N-terminal region of the
185 protein (ROGERS *et al.* 1994; GONG *et al.* 2011; GONG *et al.* 2016). We used a motif finding
186 tool (MEME; (BAILEY *et al.* 2006) to search for repetitive motifs in the coding regions of all
187 the mammalian *KRTAP1-n* sequences. This revealed that the decapeptide repeat is present at
188 the N-terminus in all mammalian *KRTAP1-n* genes we obtained (**Figure S2)**, albeit with less
189 amino acid conservation than that observed in sheep. MEME also identified nucleotide level
190 tandem copies of this repeat at the C-terminus of the protein. Furthermore, both the N- and C-
191 terminal repeats vary in copy number, within and between genomes. This copy number
192 variation is responsible for much of the length variation between *KRTAP1-n* sequences.

193 To determine the genetic relationships between of the mammalian *KRTAP1-n* genes, we
194 generated a *KRTAP1* phylogenetic tree from an alignment of our mammalian *KRTAP1-n*
195 coding region sequences. This revealed that, in most cases, the *KRTAP1* genes are more

196 related to each other within a species than to their orthologs in other species, thus exhibiting a
197 concerted evolution pattern. This manifests as clades that group by species, rather than by
198 repeat, in the phylogenetic tree (**Figure 3**). This concerted evolution pattern breaks down
199 between the most closely-related species pairs (cattle/sheep, rat/mouse, human/macaque),
200 presumably because the signal is confounded by these species having more recent shared
201 ancestry. Nevertheless, for most species there is a clear pattern of concerted evolution.

202 For concertedly evolving tandem repeat sequences such as the ribosomal RNA gene repeats,
203 homogenization occurs for the complete repeat unit, including the non-coding regions
204 (GANLEY AND KOBAYASHI 2007). To test whether the *KRTAP1* clusters display a 'whole-
205 unit' pattern of concerted evolution, we generated *KRTAP1* phylogenetic trees from multiple
206 alignments of the 5' and 3' flanking sequences of the mammalian *KRTAP1* genes.
207 Surprisingly, the phylogenies derived from these flanking sequences did not show any pattern
208 of concerted evolution, and in contrast to the coding region phylogeny, the clades in these
209 phylogenetic trees were group by *KRTAP1* repeat number, not by species (**Figure 3**). We
210 note that bootstrap support is not strong for all the clades in these phylogenetic trees, but the
211 contrast between the coding region concerted versus flanking region radiating evolutionary
212 patterns is unmistakable. Furthermore, the topology within many of the *KRTAP1* flanking
213 region clades is consistent with the reported mammalian phylogeny (refer to **Figures 2** and
214 **3**). These phylogenies were generated from multiple sequence alignments that encompass the
215 regions that align well, but phylogenies derived from sequence alignments that include poorly
216 aligned regions give qualitatively similar results (**Figure S3**). Overall, in stark contrast to the
217 coding region, the flanking regions show a phylogenetic pattern expected for normal
218 radiating evolution, and exhibit no evidence of concerted evolution.

219

220 **What is responsible for the different evolutionary patterns of the *KRTAP1* coding and**
221 **flanking regions?**

222 The difference in evolutionary pattern between the coding and flanking regions is striking,
223 hence we sought to identify the mechanism(s) responsible.

224 **Purifying selection:** Previous studies have shown that multi-gene loci undergoing birth-and-
225 death evolution can show high levels of identity within the coding region due to strong
226 purifying selection (NEI *et al.* 2000; PIONTKIVSKA *et al.* 2002). It is possible that purifying
227 selection maintains sequence identity between *KRTAP1-n* copies within a species, whilst
228 diversifying selection results in differences between species. If so, we would predict that
229 while the non-synonymous sites would show a concerted evolution pattern, the synonymous
230 sites would instead show a normal radiating pattern of evolution (resembling the flanking
231 regions).

232 To investigate this, we looked at the pattern of evolution of the synonymous sites in the
233 coding sequences compared to the non-synonymous sites. The number of KAP1 amino acid
234 changes present within and between species makes it difficult to consistently call sites as
235 synonymous or non-synonymous, so third codon positions were used as a proxy for
236 synonymous sites, and first and second codon positions were used as a proxy for non-
237 synonymous sites. We generated phylogenetic trees from multiple sequence alignments of the
238 first-second (which we refer to as "non-synonymous"), and third (which we refer to as
239 "synonymous") codon sites of the *KRTAP1-n* coding regions to test for different evolutionary
240 patterns. Surprisingly, while the non-synonymous sites displayed a pattern of concerted
241 evolution as was expected (**Figure 4A**), the synonymous sites also revealed the same pattern
242 of concerted evolution (**Figure 4B**). The concerted evolution pattern for the synonymous
243 sites seems to be stronger than that of the non-synonymous sites, as they separate sheep and

244 cattle into separate clades, and also resolve dog, elephant, and rat/mouse into separate clades
245 (**Figure 4**).

246 **Codon adaptation:** We considered whether this pattern of concerted evolution amongst the
247 synonymous sites might result from codon adaptation (LIN *et al.* 2006), as a result of
248 synonymous mutations being selected to follow changes in the favoured codons between
249 species. The *KRTAP1-n* genes display strong evidence for codon adaptation (the degree to
250 which the favoured codons for that species are used in a gene). For example, the human
251 *KRTAP1-n* genes collectively show a codon adaptation index (CAI) of 0.91 (out of a
252 maximum of 1), higher than the CAI of randomly permutated human *KRTAP1* sequences
253 (CAI=0.78). Using the *KRTAP1* coding sequence alignment used for the phylogenies
254 presented in **Figure 3**, we identified nine synonymous differences between human and mouse
255 that exhibit a concerted evolution pattern (similarity within species versus difference between
256 species). If codon adaptation can explain this pattern, these synonymous mutations should
257 change in a manner consistent with a change in codon usage preference for that amino acid.
258 Five of these mutations show the pattern expected, given the change in codon usage between
259 human and mouse (synonymous change creates the more favoured codon in the species it is
260 found in). However, four of these mutations show the opposite pattern, and most of the codon
261 usage preference changes between human and mouse are small (**Table S2**). These results
262 provide no evidence for adaptation to different codon usage preferences driving the pattern of
263 *KRTAP1* concerted evolution.

264 **Reverse transcription of *KRTAP1* mRNA:** Another potential explanation for the
265 incongruence in evolutionary pattern between the *KRTAP1* coding and flanking regions is
266 reverse transcription of *KRTAP1-n* mRNAs, followed by homologous recombination-
267 mediated replacement of a genomic *KRTAP1-n* with the reverse transcribed copy

268 (COULOMBE-HUNTINGTON AND MAJEWSKI 2007). This is feasible given that *KRTAP1-n* are
269 single-exon genes. If reverse transcription events occur, the 5' and particularly 3' flanking
270 regions should show a concerted evolution pattern that is similar to the coding region.
271 Inspection of the 5' and 3' flanking regions revealed that sequence similarity between
272 *KRTAP1-n* sequences within a genome tends to decay immediately upstream of the ATG
273 codon and downstream of the stop codon (**Figure 5**). This suggests that reverse
274 transcription/integration of *KRTAP1-n* mRNA is unlikely to explain the pattern of *KRTAP1*
275 concerted evolution, as the transcribed flanking regions of the gene would be expected to
276 'hitch-hike' with the coding regions through such a mechanism.

277 We also considered whether the *KRTAP1-n* sequences might have arisen through a pure
278 birth-and-death process by independent gene duplication events. However, we think this is
279 improbable as it would require the same number of duplications to occur in at least seven of
280 the species, and, independently, that each of these duplications would not involve any
281 flanking sequence (including promoter and terminator sequences) and have inserted into the
282 same site in each species.

283 **Gene conversion:** Finally, we considered whether gene conversion could explain the pattern
284 of *KRTAP1* repeat evolution. Gene conversion events within a genome that convert a section
285 of one repeat to the sequence of another can create homogeneity (CHEN *et al.* 2007), and the
286 degree of homogeneity depends on the relative rates of gene conversion and mutation
287 (TESHIMA AND INNAN 2004; HARPAK *et al.* 2017). Our results imply that if gene conversion
288 does occur, it is somehow restricted to the coding region. This pattern could occur if there is
289 selective pressure to maintain a degree of intra-genome homogeneity between the repeat
290 copies. If so, under the assumption that gene conversion occurs in both the coding and
291 flanking regions, those events occurring in the flanking region will not have a selective

292 advantage, while those occurring in the coding region will. Therefore, the probability of gene
293 conversion events becoming fixed in the population will be greater for events that involve the
294 coding region. There is considerable intra-genomic variation between *KRTAP1* repeats
295 (**Figure 3**), but this incomplete level of homogenization can be explained by relatively
296 infrequent gene conversion events and/or relative infrequent fixation of these events.
297 Therefore, the sequence features of the *KRTAP1* repeats that we document here can all be
298 accounted for by gene conversion coupled with selection.

299

300 **Evidence for gene conversion events in the *KRTAP1-n* repeats**

301 Inspection of the *KRTAP1* coding region multiple sequence alignment provides evidence for
302 tracts of gene conversion. Specifically, sites where there are mutations that are shared
303 between copies within a species, but that differ between species, are frequently clustered
304 together rather than scattered throughout the gene (**Figure 6**). Such patches of homogeneity
305 are expected if there has been occasional, short-tract gene conversion events. The patches we
306 observe are small, but are within the expected range for mammalian gene conversion events
307 (CHEN *et al.* 2007). In addition, we collected population polymorphism data for *KRTAP1-n*
308 sequences in sheep, as comprehensive sequence variation data are scarce in other species. For
309 many of the sites that are polymorphic, the polymorphism is shared across some, or all, of the
310 *KRTAP1-n* sequences (**Figure 7**). While we cannot rule out independent mutation events in
311 each *KRTAP1* copy, we think that gene conversion is a more parsimonious explanation for
312 this observation, particularly for the polymorphisms at synonymous sites. Gene conversion
313 has also previously been suggested as an explanation for the pattern of polymorphism in the
314 ovine *KRTAP1* genes (ROGERS *et al.* 1994). Collectively, our results suggest that the unusual
315 evolutionary pattern of the *KRTAP1* repeats, where the coding region evolutionary dynamics

316 are uncoupled from those of the flanking region, is the result of occasional short-tract gene
317 conversion events that are selected for in the coding region but not the flanking regions, and
318 that drive partial homogenization.

319

320

321 **DISCUSSION**

322 Here we have shown that *KRTAP1-n* genes are conserved as a block of four tandem repeats in
323 mammalian species, and this suggests they derive from a relatively ancient gene-
324 amplification event or events that probably pre-date mammalian speciation. These four
325 tandem copies display a strong pattern of concerted evolution in the coding regions, yet the
326 regions flanking show a normal radiating pattern of evolution. We suggest that this
327 dichotomous pattern of evolution is not the result of purifying selection acting to retard
328 changes to the amino acid sequence, but instead results from short gene conversion tracts that
329 periodically homogenize sequences between the four *KRTAP1* genes.

330 The role of gene conversion is supported by two key pieces of evidence: 1) unique amino
331 acid tracts that are shared by KAP1 copies within a species, but are unique to that
332 species/group of related species; and 2) the possession of shared nucleotide variants between
333 *KRTAP1* gene copies in sheep populations. These results extend previous reports of
334 homogenization via ongoing short-tract gene conversion events in other protein coding genes
335 (NOONAN *et al.* 2004; LAMPING *et al.* 2017).

336 We propose that gene conversion is being utilized as an unusual form of purifying selection
337 that prevents accumulation of too much divergence between *KRTAP1* gene copies. We
338 speculate that homogeneity of the *KRTAP1* coding sequences is beneficial as it enables the

339 production of more homogenous components of the hair and wool fibre matrix, and thus
340 potentially facilitates better associations with the keratin intermediate filaments. We cannot,
341 however, rule out the possibility that individual *KRTAP1* repeats might have functional
342 differences, the signal of which is overwhelmed by the concerted evolution signal from the
343 majority of the gene. However, we note that, particularly in dogs, some of the *KRTAP1-n*
344 genes are very similar in sequence. Therefore, we favour the explanation that *KRTAP1*
345 concerted evolution results from ongoing, stochastic gene conversion events coupled with
346 selection within the coding region against inter-repeat heterogeneity.

347 Purifying selection is evident in the *KRTAP1-n* coding regions, as the rate of synonymous
348 change is about twice that of the non-synonymous rate (**Figure 4**). While this may seem to
349 contradict the similarity in the synonymous and non-synonymous concerted evolution tree
350 topologies, it can be simply explained by purifying selection acting on residues that are
351 conserved between species, and thus not contributing to the synapomorphies that influence
352 the tree topologies. Any gene conversion events that homogenize unfavourable amino acids
353 will be selected against, thereby preventing deleterious mutations from spreading between
354 copies. However, this same process also allows tolerable and advantageous amino acid
355 changes to sweep through the copies (DOVER 1982). The *KRTAP1-n* sequences from closely
356 related species (i.e. human and macaque, rat and mouse, sheep and cattle) were not separated
357 into different clades for most of the phylogenetic trees we generated (**Figures 3 and 4**). This
358 suggests that the rate of homogenization is relatively slow, and insufficient to drive
359 substantial homogeneity over the evolutionary time frames separating these species pairs. In
360 this context, the shared polymorphisms that we observe in sheep (that are evidence for gene
361 conversion events) are likely intermediate stages in the accumulation of homogenized
362 *KRTAP1-n* sequences.

363 The sharp border between a concerted evolution pattern in the coding region and a radiating
364 evolution pattern in the immediate flanking regions is striking. This can partially be explained
365 by the selection for gene conversion events within the coding region, as we have proposed.
366 However, it is intriguing to speculate that this may also be a consequence of differential
367 expression between the *KRTAP1* genes that is mediated by copy-specific differences in the
368 regulatory regions. Although not direct, some evidence for differential regulation of
369 *KRTAP1-n* gene expression was found in two transcriptome studies looking for differentially
370 expressed genes (FAN *et al.* 2013; CHANG *et al.* 2014). If the *KRTAP1-n* genes do have
371 functionally distinct roles, gene conversion events in the *KRTAP1* regulatory regions that
372 perturb their differential regulation may be maladaptive and therefore selected against. Thus,
373 selective pressure for coding region homogeneity versus regulatory region diversity, coupled
374 with ongoing gene conversion, may be a powerful way to achieve the dichotomy in
375 evolutionary patterns we observe. Clearly, a better understanding of the transcriptional
376 regulation of the *KRTAP1* genes is required to address this hypothesis.

377 Gene conversion is frequently viewed through the lens of impeding sub-functionalization of
378 gene duplicates. This view is consistent with the well characterized case of the opsin gene
379 duplicates in primates, where there is a much stronger signal of gene conversion/concerted
380 evolution in the introns, than in the exons (SHYUE *et al.* 1994; HIWATASHI *et al.* 2011). The
381 interpretation is that selection has largely rejected gene conversion events that include the
382 coding (exon) regions, whilst allowing those occurring in the non-coding (intron) regions to
383 spread in the population (SHYUE *et al.* 1994). This is the opposite of what we observe, and
384 illustrates how gene conversion and selection can intersect to produce a constellation of
385 evolutionary patterns: homogenization of the non-coding but not the coding regions in the
386 opsin paralogs (SHYUE *et al.* 1994); homogenization of the coding but not the non-coding

387 regions in the *KRTAP1* genes (this study); and homogenisation of both coding and non-
388 coding regions equally in the ribosomal RNA gene repeats (GANLEY AND KOBAYASHI 2007).

389 The extent to which gene conversion acts to homogenize gene duplicates remains
390 controversial (GAO AND INNAN 2004; CASOLA *et al.* 2012; HARPAK *et al.* 2017). Furthermore,
391 even in examples where recurrent gene conversion events can be detected, they are often not
392 sufficient to produce a strong concerted evolution pattern (PETRONELLA AND DROUIN 2011;
393 PETRONELLA AND DROUIN 2014). There are two potential explanations for why such a strong
394 pattern of concerted evolution is observed in the case the *KRTAP1* genes, despite the
395 relatively high levels of divergence between copies. First, unlike many of the examples that
396 have aroused controversy (GAO AND INNAN 2004; CASOLA *et al.* 2012; HARPAK *et al.* 2017),
397 the *KRTAP1-n* repeats are tandemly-arranged. Proximity effects as a consequence of tandem
398 arrangement may increase the chances of unequal alignment of the repeats during DNA
399 repair-based homologous recombination compared to dispersed repeats, and thus may
400 increase the chances of conversion events. However, this does not explain examples where
401 tandemly repeated paralogs do not show a strong concerted evolution pattern (NEI *et al.* 2000;
402 PERINA *et al.* 2011). A second explanation relates to the imperfect decapeptide tandem repeat
403 motif found in the coding region. Variation in the copy number of decapeptide repeats
404 between *KRTAP1* genes is possibly the result of unequal recombination (LIAO AND WEINER
405 1995; GANLEY AND SCOTT 1998; MORRILL *et al.* 2016). If so, the *KRTAP1* genes may
406 harbour a recombination hotspot that drives both decapeptide repeat copy number variation
407 and gene conversion at higher than average levels.

408 Repeats are ubiquitous denizens of eukaryote genomes, where they exist in different forms
409 (coding, non-coding) and organizations (tandem, dispersed). Our results add to the growing
410 list of examples that illustrate how different molecular and evolutionary processes can

411 impinge on repeats to structure their sequences and create distinctive patterns of evolution
412 (SHYUE *et al.* 1994; NOONAN *et al.* 2004; GANLEY AND KOBAYASHI 2007; STORZ *et al.* 2007;
413 HIWATASHI *et al.* 2011; LAMPING *et al.* 2017). However, it is unclear how widespread these
414 sorts of evolutionary dynamics are for eukaryotic gene repeats, largely because the patterns of
415 evolution have not been investigated for the vast majority of multi-gene families. The
416 increasing availability of high quality genome sequences for a wide range of eukaryotes puts
417 us in an excellent position to determine, on a much more systematic and wide-ranging basis,
418 the patterns of repeat sequence dynamics and evolution. This will, in turn, make it clear
419 whether the impact of recombination on the *KRTAP1*s is unusual, or highlights a common
420 mechanism to finely scale patterns of homogeneity and divergence between repeat copies
421 over time.

422

423

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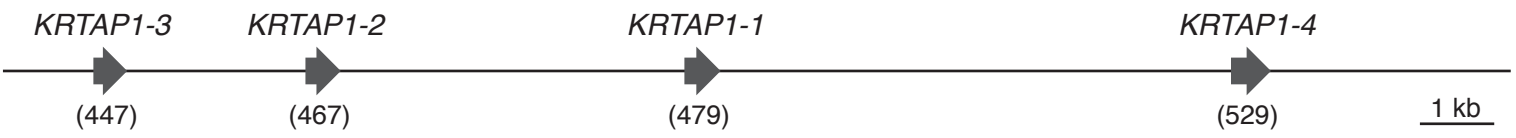

Figure 1. Tandem repeat organization of the keratin associated protein-1 (*KRTAP1*) genes
The organization of mammalian *KRTAP1* genes is illustrated by the arrangement found in sheep. The four *KRTAP1-n* paralogs are represented by arrows that indicate the direction of transcription. Diagram is drawn to scale, with *KRTAP1-n* lengths bracketed below the genes. These repeats are numbered *KRTAP1-1, 3, 4*, and *5* in human.

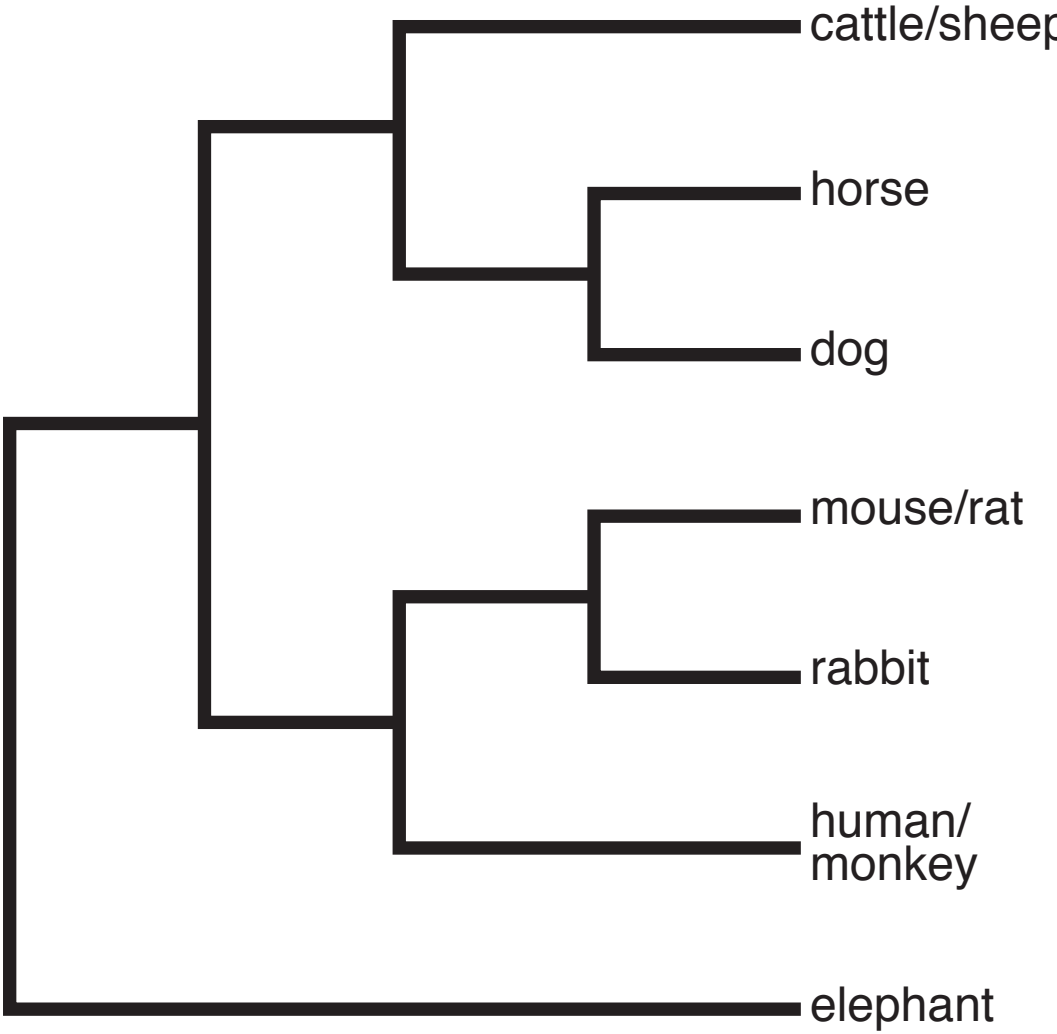

Figure 2. Mammalian *KRTAP1-n* gene phylogenetic relationships
Representative phylogenetic tree illustrating the relationships between the *KRTAP1-n* genes in the species used in this study. Branch lengths are not to scale. The phylogeny is adapted from that presented in McCormack et al. (2012).

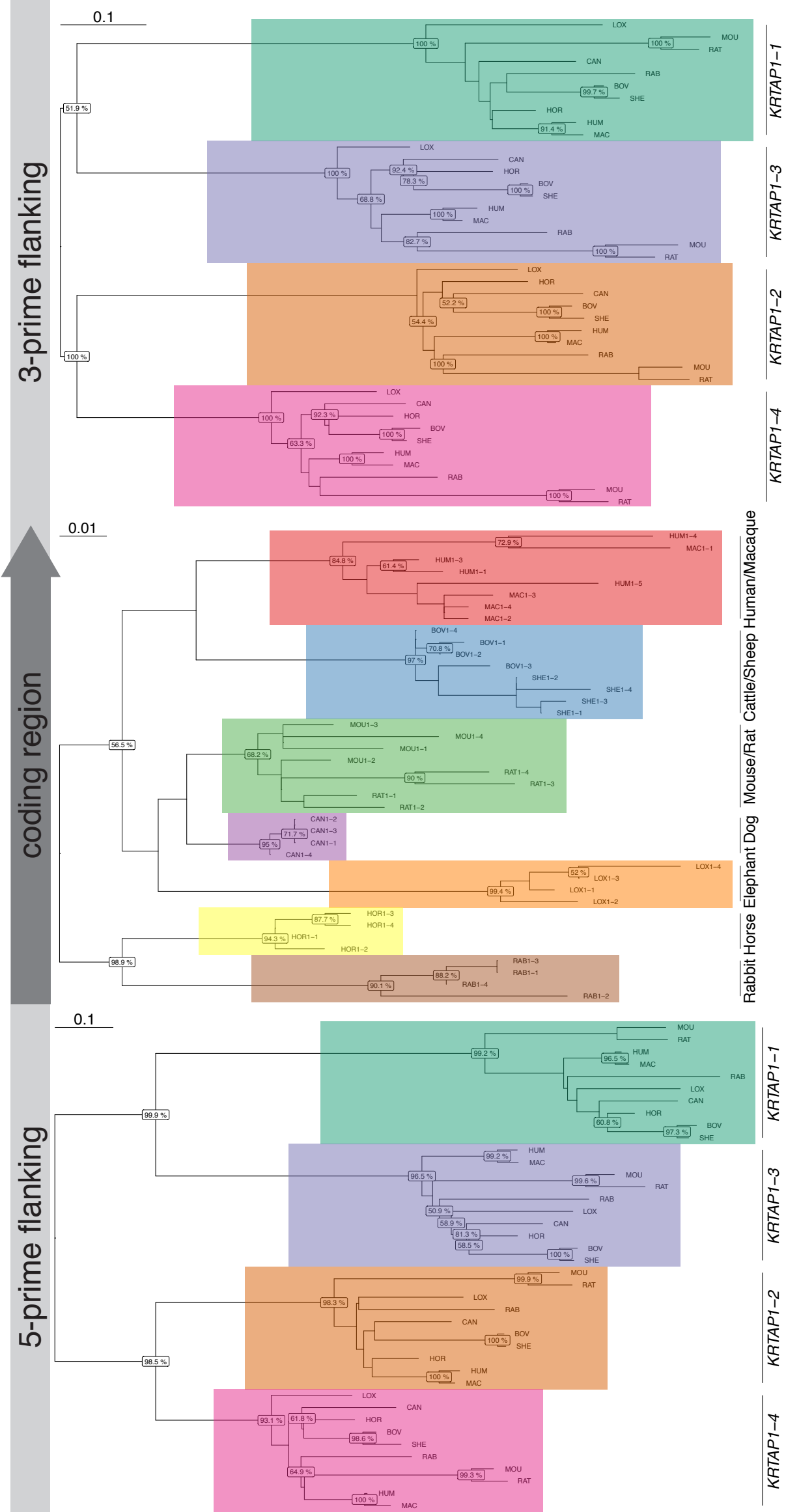

Figure 3. Phylogenetic trees of *KRTAP1-n* coding and flanking region sequences
Phylogenetic trees were constructed for the mammalian *KRTAP1-n* 5' flanking region, coding region, and 3' flanking region using PhyML. The species are indicated by three-letter abbreviations. The number following this for the coding regions indicates the *KRTAP1-n* gene name. The major clades within the trees are indicated by coloured boxes. The 5' and 3' flanking region phylogenies group by repeat number, while the coding region phylogeny tends to group by species. Numbers on nodes indicate bootstrap supports over 50%, and substitution rates are indicated at the top left. Human *KRTAP1-n* gene names have been altered for consistency with other species.

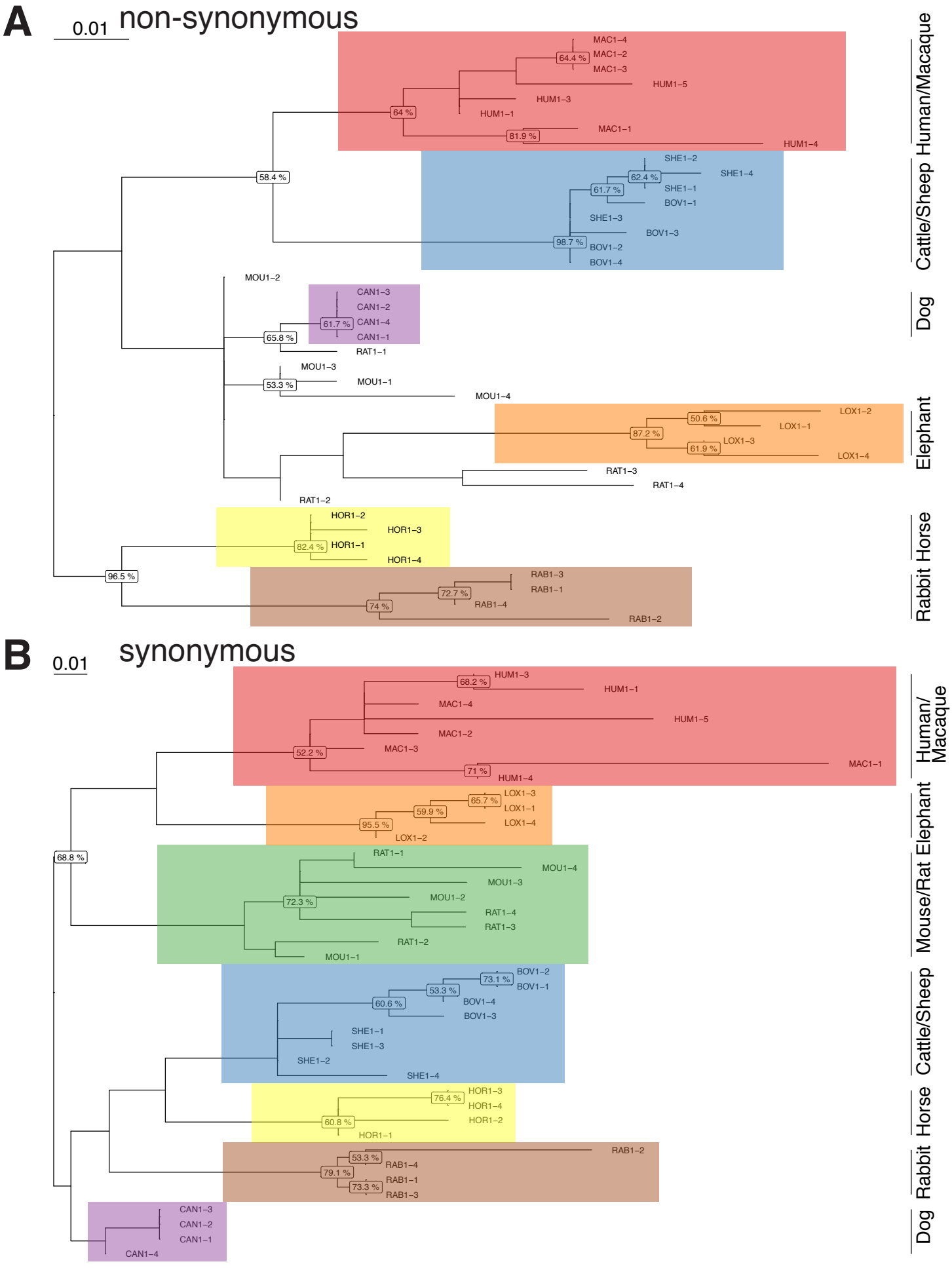

Figure 4. The *KRTAP1-n* concerted evolution pattern is not explained by purifying selection
Phylogenetic trees were constructed for the 1st and 2nd codon positions ("non-synonymous"; **A**), and the 3rd codon position ("synonymous"; **B**), as per **Figure 3**. The major clades in both phylogenies tend to group by species, with this concerted evolution pattern being stronger for the synonymous phylogeny. Numbers on nodes indicate bootstrap supports with values over 50%, and substitution rates are indicated at the top left.

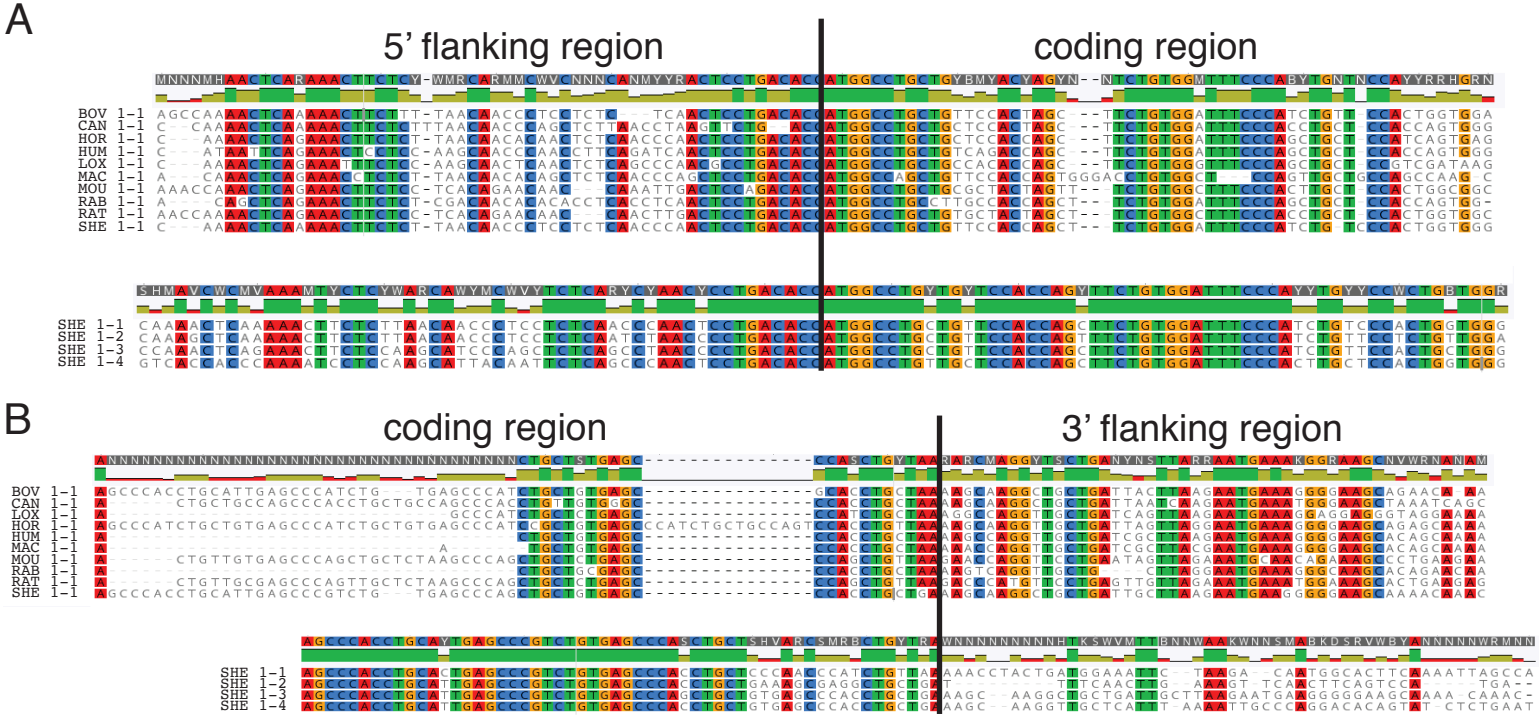

Figure 5. The switch between concerted and radiating evolution patterns is located close to the start/stop sites

A) Alignment of the region flanking the *KRTAP1-1* gene start site. The boundary between the 5' flanking and coding regions is marked by a vertical line (followed by the ATG). Underneath is an alignment of the same region for all four *KRTAP1-n* sequences from sheep. Mismatches have a white background, conservation is indicated graphically above each alignment, and consensus sequences are shown at the top. **B)** As in **(A)**, except the region flanking the stop site is shown, with the vertical line marking the boundary between the coding and 3' flanking regions (preceded by the stop codon).

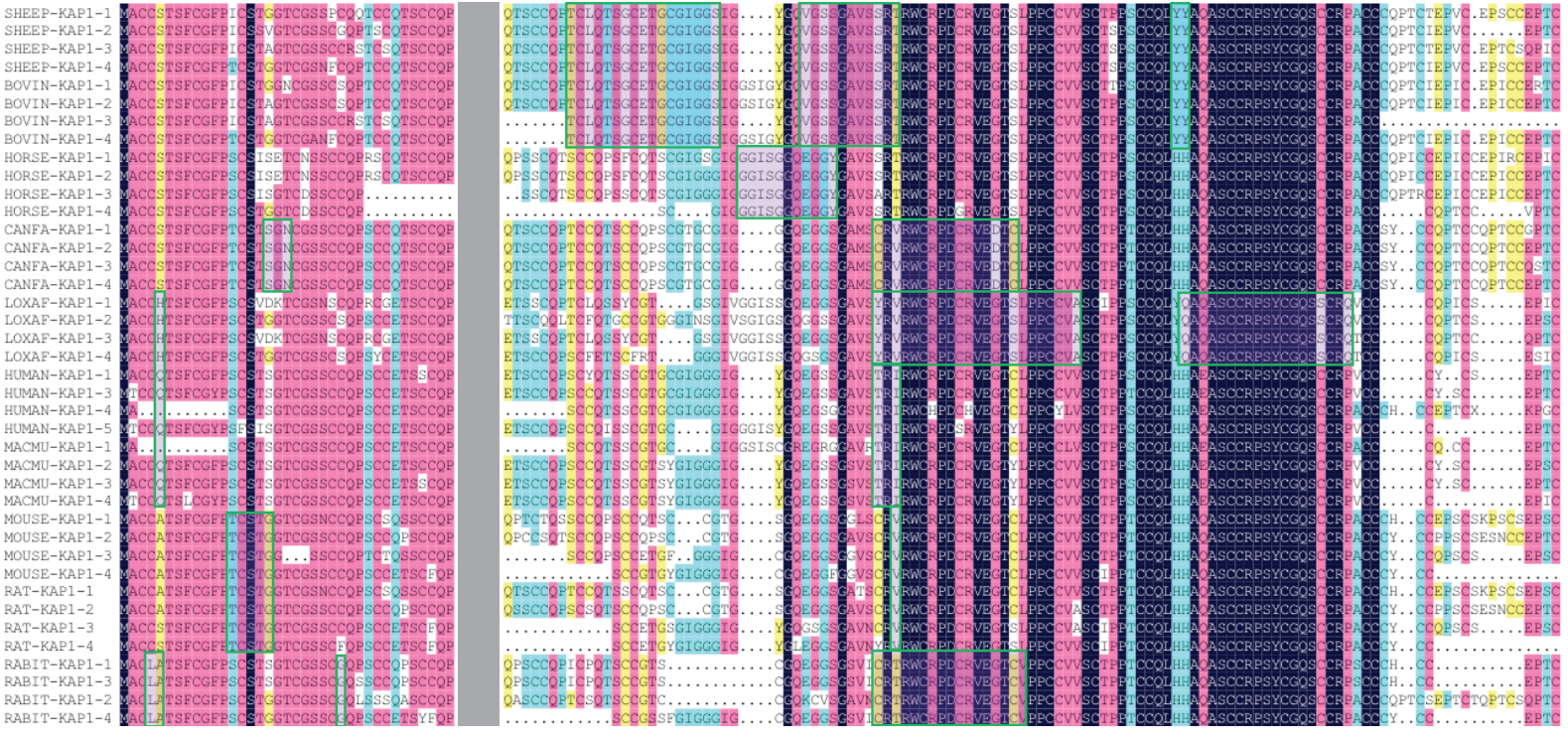

Figure 6. Evidence for short gene conversion tracts between *KRTAP1-n* sequences within species
Alignment of KAP1 amino acid sequences from the ten mammalian species. Amino acid tracts boxed in green represent sequences unique to a species or related species pairs. The grey vertical box represents the conserved decapeptide repeat sequences (which have been removed). Dots represent gaps in the alignment.

```
KRTAP1-1  ATGGCCTGCTGTTCCACCAGCTTCTGTGGATTTCCCATCTGTYCCACTGGTGGGACCTGTGGCTCCAGTCCCTGCCAGCMGACCTGCTGC <30 bp repeat>
KRTAP1-2  --------------------T----------------------T--T---T---A---------------CTG-G------CA----C---- <30 bp repeat>
KRTAP1-3  ------------------------------------------Y------C------------------CTG-----GATCA------A-T <30 bp repeat>
KRTAP1-4  --------T--C-------------------------CT--CT--------------------T----ACTTT-------CA--------- <30 bp repeat>

KRTAP1-1 CAGACCAGTGGCTGTGAGACSGGCTGTGGCATTGGTGGCAGCATTGGYTATGGCCAGGTGGGTAGCAGCGGAGCTGTGAGCAGCCGCACCAGGTGGTGCCGCC
KRTAP1-2 ---------------------V-----------W--------------C----R---------------Y-----------------------------------
KRTAP1-3 -----------Y---R----S----------------------Y---C-----------------------------------------------R----------
KRTAP1-4 ---------------------B-----------W----------Y--RC-------------------------------------------------------

KRTAP1-1 CTGACTGCCGCGTGGAGGGCACCAGCCTGCCWCCCTGCTGYGTGGTGAGCTGCACAYCCCCGTCCTGCTGCCAGCTGTACTATGCCCAGGCCTCCTGCTGCCG
KRTAP1-2 --------------------------------T--------Y---------------T------------R-----------------------------------
KRTAP1-3 ----------------------------R--T--------Y---------------Y-----------------------------------------------
KRTAP1-4 --------------------------------T--------Y---------------T-----------------------------------------------

KRTAP1-1 CCCATCCTACTGTGGACAGTCCTGCTGCCGCCCAGCCTGCTGCTKCCAGCCCACCTGCAYTGAGCCCRTCTGTGAGCCCAGCTGCTGTGAGCCCACCTGCTGA
KRTAP1-2 ----------------------------------------------G--------------T-------G------------S----...............---
KRTAP1-3 ----------------------------------------------G--------------Y-------G------------C-----CCC-A----T-M-T-A-
KRTAP1-4 ----------------------------------------------K--------------T-------R------------S--------------R--------
```

Figure 7. Shared polymorphisms between *KRTAP1-n* sequences in sheep

Alignment of the four sheep *KRTAP1-n* coding region sequences. Dashes represent nucleotides identical to the top sequence, and dots represent gaps. The 30 bp repeats are not shown, as the insertion/deletion positions cannot be precisely determined. Shared nucleotide substitutions between repeat copies are highlighted in red.