Examination of Hemiplasy, Homoplasy and Phylogenetic Discordance in Chromosomal Evolution of the Bovidae

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Abstract.—Robertsonian chromosomal fusions predominate in shaping the genomes of many species of Bovidae. These and other cytogenetic data (from 52 taxa representing 51 species and 9 tribes of Bovidae) were (i) examined for usefulness in defining phylogenetic relationships and (ii) subsequently mapped to a consensus tree based on mitochondrial and nuclear DNA gene sequences with divergence dates of the corresponding species calculated from cytochrome b sequences. This permitted persistence time estimates for the various rearrangements. The chromosomal data resulted in an unsupported higher-level topology, but with recognition of the monophyly of some genera and tribes within Bovidae. The distribution and temporal spread of character states on the species tree is suggestive of a restricted role for hemiplasy (the retention of an ancestral chromosomal polymorphism through multiple speciation events) and for introgression (resulting from secondary contact among taxa), processes that can potentially lead to phylogenetic discordance. We conclude that the most probable interpretation for these data is that genuine karyotypic homoplasy predominates, but that hemiplasy (and/or introgression) is a realistic hypothesis for the observed patterns of several shared characters in Bovidae. [Bovids; chromosomes; evolution; hemiplasy; homoplasy; hybridization; lineage sorting; systematics.]

One of the most intriguing aspects of chromosomal evolution within Bovidae (antelope, cattle, sheep, and goats) is the high number of Robertsonian (Rb) fusions characterizing the karyotypes of many species (Wurster and Benirschke 1968; Effron et al. 1976; Buckland and Evans 1978; Gallagher and Womack 1992; Gallagher et al. 1994; Vassart et al. 1995). Recorded chromosome numbers vary from $2n = 30$ in gazelles to $2n = 60$ in a range of species within the tribes Aepycerotini, Hippotragini, Oreotragini, Cephalophini, and Bovini. Although taxon representation is often relatively sparse (the exceptions being the Bovini, Antilopini, Reduncini, and Tragelaphini), banding (G- or Q-banding) and/or fluorescence in situ hybridization (FISH) data are available for representative members of the eight recognized superfamilies (Wilson and Reeder 2005) and nine tribes. However, in spite of the availability of these data, there has, as yet, been no attempt to examine their usefulness in a comprehensive framework that assesses correspondence, if any, to bovid sequence-based molecular phylogenies (e.g., Allard et al. 1992; Hassanin and Douzery 1999; Matthee and Davis 2001 among others) and to determine to what extent these chromosomal markers define monophyletic lineages. This latter aspect is particularly important because Rb fusions are often polymorphic within species (Wurster and Benirschke 1968; Vassart et al. 1995; Kingswood et al. 1998; Rubes et al. 2007) and it has been argued that they are phylogenetically uninformative (Robinson et al. 1997).

In spite of the large number of molecular and morphological studies on Bovidae (Fernandez and Vrba 2005 and references therein), a definitive phylogeny remains elusive echoing Simpson’s (1945) early view that Bovidae is one of the most problematic mammalian groups to classify. Factors that have contributed to taxonomic and phylogenetic uncertainty include morphological convergence, a rapid radiation, and the questionable monophyly of the group (Gatesy et al. 1992). For example, Vrba (1979) and Janis and Scott (1988) among others have documented considerable morphological convergence in Bovidae and other ruminants obscuring true evolutionary relationships. Even with this, however, there is broad consensus for recognition of two distinct clades within Bovidae—the Bovinae, and a larger, more diverse assemblage, the Antilopinae (Hassanin and Douzery 1999; Hassanin and Douzery 2003; Matthee and Davis 2001)—a dichotomy first proposed by Kingdon (1982, 1997) based on differences in thermoregulation and gland structure. Although there is slight disagreement on the number of tribes, a broad consensus suggests nine within Antilopinae (Aepycerotini, Alcelaphini, Antilopini, Caprini, Cephalophini, Hippotragini, Neotragini, Oreotragini, and Reduncini) and three within Bovinae (Bovini, Boselaphini, and Tragelaphini—Hassanin and Douzery 1999; Hassanin and Ropiquet 2004; Ropiquet et al. 2009).

Given the temporal compaction of the subfamilial divergences, three potential outcomes could be predicted with respect to the chromosomal rearrangements that distinguish modern bovid karyotypes: (i) where short internodes characterize the diversification of tribes few shared derived chromosomal rearrangements (synapomorphies) would exist among them resulting in a lack of resolution at this level; (ii) the appearance of homoplastic characters in unrelated lineages would underscore the propensity for Rb fusions to occur convergently; (iii) the presence of long branches prior to the rapid diversification of species within tribes could result in the accumulation of numerous autapomorphies (and convergent changes i.e., homoplasies) along
the common branch and, in instances where species radiation is rapid, the polymorphic state could persist over several speciation nodes leading to homoplasy (Avise and Robinson 2008; Robinson et al. 2008) and spurious phylogenetic interpretations. Here, we examine the potential influences of homoplasy and hemiplasy on bovid chromosomal evolution, processes that can obscure the actual history of species divergence, and the degree to which these possibilities may materially affect this karyotypic data set.

METHODS

Species Surveyed

We surveyed the bovid comparative cytogenetic literature limiting our comparisons to studies that relied on banding (G- and Q-banding) and/or FISH, to instances where the chromosomal rearrangements were defined with respect to the cattle standard (ISCNDB 2001), and, lastly, to species for which cytochrome b sequences were available (see below). Species included in our analysis and source references are presented in Table 1. These should be consulted for karyotype descriptions and correspondence with the cattle standard.

Although we attempted to be exhaustive in our coverage, where two reports on the same species were available and these differed (Damalis cus lunatus, Gallagher and Womack 1992 and Kumamoto et al. 1996; Taurotragus derbianus, Rubes et al. 2008 and Nguyen et al. 2008; Ovis nivicola and O. ammon, Huang et al. 2005 and Bunch et al. 2006), we included only a single representative karyotype of each to constrain homoplasy in the data. Our selection was based on FISH data in the case of Taurotragus derbianus (it and T. oryx have identical karyotypes, Rubes et al. 2008), consensus in the case of the caprine species (Bunch et al. 2006 and references therein), and the more recent publication date and greater species representation in the case of D. lunatus (Kumamoto et al. 1996).

Characters

In total 155 characters were analyzed for 52 taxa (51 species—we retained Bos javanicus birmanicus and B. j. javanicus as separate taxonomic entities) representative of all nine tribes in Bovidae. Chromosomal rearrangements (characters) were scored for their presence or absence (characters states) in the ingroup taxa based on comparison with B. taurus (which is thought to have retained the bovid ancestral karyotype with 58 autosomal acrocentric chromosomes) following Nguyen et al. (2008), and the principles outlined in Dobigny et al. (2004). In addition to the Rb fusions (centromere–centromere) detected in the survey, we also scored the presence versus absence of centromere–telomere translocations, reciprocal translocations, differences in X chromosome morphology and gonosome, and autosome translocations. Transformations affecting the X chromosomes have been coded as described by Nguyen et al. (2008). Missing data were coded as “?” and the character matrix deposited as online Appendix 1, Dryad: http://dx.doi.org/10.5061/dryad.8332.

Phylogenetic Analysis

We followed two approaches in our analysis of the chromosomal data. First, we examined the full data set by parsimony rooted on Cervus nippon (Cervidae). Analyses were conducted with PAUP 4.0b10 (Swofford 2003) using the tree bisection–reconnection branch swapping algorithm. Support for phylogenetic associations was determined using bootstrap percentages (BPs) calculated after 100 replicates under the closest stepwise addition option.

Second, we scrutinized the chromosomal data set more fully to determine whether the effects of homoplasy were sufficiently pronounced to obscure the information content of the relatively few parsimony informative chromosomal characters. Although it is possible to construct a mtDNA gene tree using cytochrome b sequences for all taxa in our chromosomal data set (Table 1), the exclusive reliance on mtDNA data (and its susceptibility to introgression) necessitated a multilocus approach to provide a species tree that is well sampled and that accurately places the species with respect to each other. We therefore constructed a consensus tree using published mitochondrial and nuclear trees that are available in the literature (Hassanin and Douzery 1999; Mathee and Davis 2001; Hassanin and Ropiquet 2004; Willows-Munro et al. 2005; Ropiquet and Hassanin 2006; Hassanin and Ropiquet 2007; Marcot 2007; Nijman et al. 2008; Ropiquet et al. 2009). Nodes are based on the supertree analysis of seven molecular markers by Ropiquet et al. (2009) that involved three different measures of robustness and reliability and the outcomes from the supermatrix studies by Marcot (2007) for the Reduncini, Willows-Munro et al. (2005) for the Tragelaphini, and Nijman et al. (2008) and Hassanin and Ropiquet (2004, 2007) for Bovini. In all these, robustness of the nodes was determined by bootstrap or posterior probabilities values. Source references for each species used in our consensus tree are presented in Table 2. Moreover, although well-supported short internodes can be misleading in supermatrix analyses (Ropiquet et al. 2009), our conclusions are not biased by the dominance of one marker, and there is a good correlation between the robustness of the nodes of the consensus tree and the reproducibility of the results in the independent analyses of the various data sets.

Mapping of character evolution on the consensus tree followed the likelihood reconstruction method in Mesquite version 1.12 (Maddison W.P. and Maddison D.R. 2009). This was used to determine the state assignment at each ancestral node that maximizes the probability of obtaining the observed states in the terminal taxa under the specified model of evolution (MKI model, in this study). Potential hemiplasies were also manually checked to ensure that they met the prerequisites of short internodes and realistic persistence times (see below).
Table 1. Species included in the phylogenetic analysis and source references for the chromosomal and sequence data

|Molecular Dating|

Molecular divergence dates were calculated from cytochrome b sequences for all species in the consensus tree (see Table 1 for accession numbers). This was done using the uncorrelated relaxed clock method implemented in BEAST 1.5.2 (Drummond et al. 2006) that employs a Bayesian Markov chain Monte Carlo (MCMC) to coestimate topology, substitution rates, and node ages. MrModeltest 2.2 (Nylander 2004) was employed to select the model of DNA substitution that best fits the data. GTR + I + G was selected based on the Akaike information criterion. The analysis implemented a Yule branching rate prior with rate variation across branches assumed to be uncorrelated and log-normally distributed. Each final MCMC chain was run for 1 million generations (burn-in 10%) sampling trees at every 100 generations. Examination of MCMC samples using TRACER (Rambaut and Drummond 2007) suggested that the independent chains were each adequately sampling the same probability distribution. Divergence dates and 95% confidence intervals were calculated for each node. Prior estimates of the divergence dates for the Bovidae follow Drummond et al. (2006, 2007) and Ho (2007). We assume that a normal distribution (which allows divergence dates to vary symmetrically and with soft bounds) centered at 19 myr with a standard deviation of 0.61 myr to accommodate the 18–20 myr range suggested by fossil record estimates (Solounias et al. 1995; Gentry et al. 1999; Vrba and Schaller 2000).
Table 2. Species included in the construction of the consensus tree.

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RESULTS AND DISCUSSION

Parsimony Analysis of Chromosomal Characters

(i) Higher-level relationships: Our chromosomal binary matrix comprised 155 characters of which 86 were parsimony informative. A heuristic search with equal weighting found 5000 equiparsimonious trees of 201 steps with a consistency index = 0.7761 and a retention index = 0.7772. The BP tree is presented in Fig. 1. As is evident, parsimony analysis of the chromosomal data set results in an unsupported higher-level topology that clearly underscores the limitation of chromosomal characters, in particular Rb fusions, in resolving these relationships within Bovidae. There is no cytogenetic support for the Bovinae/Antilopinae dichotomy in the parsimony analysis—an association that is invariably retrieved by molecular and morphological investigations—nor for resolution among tribes. This reflects the overwhelming effects of chromosomal homoplasy at the deeper nodes. The rapid contemporaneous tribal radiation that followed the Bovinae/Antilopinae split (Gentry 1994; Kingdon 1997; Hassanin and Douzery 1999; Matthee and Davis 2001; Hassanin and Douzery 2003; Fernández and Vrba 2005; Ropiquet and Hassanin 2005) most likely allowed insufficient time for chromosomal synapomorphies to
establish and/or to the obliteration of characters by idiosyncratic lineage sorting. Published molecular divergence dates (Hassanin and Douzery 1999) show that the diversification occurred in a narrow temporal window in the mid-Miocene—the major lineages in Bovinae and Antilopinae diversified during the middle Miocene (16.4–11 Ma)—all of which are broadly consistent with the dating obtained from cytochrome b sequences analyzed in our investigation (Fig. 2).

(ii) Generic relationships: The parsimony tree provides limited support for the monophyly of genera and some tribes on cytogenetic grounds. This included recognition of Bubalus (BP = 90), Damalisus (BP = 100), and Nanger (BP = 97). A sister relationship for Oryx dammah and O. gazella is weakly indicated (BP = 43; Hippotragini) as is the monophyly of Tragelaphini (Tragelaphus + Taurotragus; BP = 43) and Reduncini (BP = 53). The monophyly of Ovis is moderately supported (BP = 64). The phylogenetic positions of several taxa whose systematic positions are controversial (i.e., Neotragini represented by Neotragus, Oreotragini by Oreotragus, Cephalophini represented by Philantomba, and Aepycerotini by Aepyceros) are not supported by the chromosomal data.

Mapping Chromosomal Characters to the Consensus Tree

The distribution of homoplastic chromosomal characters across lineages within Bovidae was overwhelmingly evident (online Appendix 2, doi:10.5061/dryad.8332). Of the 155 chromosomal characters in our complete data set, 68 are autapomorphies, 41 are exclusive synapomorphies, 36 are strictly homoplastic, 4 are ambiguous, and 6 are hemiplasic when mapped to the tree (online Appendix 1, doi:10.5061/dryad.8332). Chromosomal support for some of the “consensus” clades within Bovidae includes the diagnostic reciprocal translocation BTA9;14 that potentially defines Antilopinae, and within this subfamily, Rb 3;19 that underpins the Alcelaphini. An autosome/gonosome
translocation (X;5) is a synapomorphy for a clade that groups Gazella + Nanger + Eudorcas + Antilope (online Appendix 1, doi:10.5061/dryad.8332). In similar fashion, an inversion of the X chromosome defines the monophyly of Bovinae, an X centromeric shift the Bovina (Nguyen et al. 2008 and references therein), and BTA13;Y has previously been identified at the base of Tragelaphini (Rubes et al. 2008).

**Chromosomal Evolution within Bovidae**

Approximately 27% of all characters detected by our phylogenetic review of chromosomal rearrangements that have shaped the karyotypes of sheep, cattle, goats, and antelope are potentially homoplastic, the majority comprising Rb fusions. This contrasts with gonosome/autosome translocations (thought to be significantly underdominant, Ashley 2002) which, with one exception (Y;13 in Tragelaphini), define the groups within which they occur (online Appendix 1, doi:10.5061/dryad.8332). These findings raise several interesting questions. For example, what predisposed certain lineages to undergo rampant Rb fusions (and to a lesser extent other rearrangements) and not others? Is there evidence to suggest that habitat/population fragmentation may have been important in fixing rearrangements? Could introgressive hybridization among species that have not evolved reproductive isolation contribute to the apparent homoplasy in the bovid chromosomal tree and what role, if any, could hemiplasy (Avise and Robinson 2008) play in explaining some of the patterns retrieved by our study?

Rb or centric translocations involving the fusion of two nonhomologous acrocentric or telocentric chromosomes at their centromeres (producing a single biarmed product with distinguishable internal centromere) are...
frequent in ruminant chromosome evolution and particularly so in Bovidae (Buckland and Evans 1978; Gallagher and Womack 1992; Gallagher et al. 1994, 1999; Vassart et al. 1995 among others). Although by no means a universal characteristic (e.g., the Bovina are almost exclusively 2n = 60), multiple fusions have been responsible for impressive diversity of karyotypes detected in most other lineages that is best exemplified by the Antilopini with 2n = 31–60. Several hypotheses have been advanced to explain the formation of Rb fusions in mammals usually invoking the organization of pericentromeric satellite DNA (Modi et al. 1996; Chaves et al. 2003 among others) and nonallelic homologous recombination at meiosis (Page et al. 1996; Ferguson-Smith and Trifonov 2007). Breakpoints are considered to occur in regions of segmental duplication (Kehrer-Sawatzki and Cooper 2008) and high concentrations of repetitive elements (Ruiz-Herrera et al. 2006). Therefore, even though the same brachial combinations characterize Rb fusions in different taxa, the precise position of a breakpoint may vary (reflecting independent or de novo origins for these rearrangements), thus giving credence to the suggestion that these are, in many instances, true homoplasies. Whatever the precise mechanism, however, most of the 156 chromosomal rearrangements detected in our survey of 52 taxon-specific karyotypes are centric fusions (141 of 156 characters). This contrasts with the high occurrence of species with karyotypes comprising 2n = 58 acrocentric autosomal chromosomes that correspond fully in brachial homology to those that deviate from this diploid number, leading to suggestions that this may represent the ancestral condition for Bovidae (Wurster and Benirschke 1968; Buckland and Evans 1978; Gallagher et al. 1994). Although the reason(s) for some lineages to undergo chromosomal reorganization and others not remains a central enigma in chromosomal evolution (possibilities include the release of transposon activity, Gray 2000 and references therein), it is nonetheless possible to suggest cogent scenarios that would explain the fixation of chromosomal mutations within bovid species should they occur and the likely reasons for their subsequent phylogenetic discordance.

Hassanin and Douzery (1999) demonstrated three main episodes of bovid radiation. The first involved vicariance that resulted in the isolation of Bovinae in Eurasia and Antilopinae in Africa at the end of the early Miocene. The second was the tribal radiation during mid-Miocene that gave rise to the majority of tribes in Bovinae and Antilopinae which is consistent with paleoenecological trends in the late Cenozoic that suggest bovids radiated independently in southern Asia and Africa 16.0–13.5 Ma (Behrens-Meyer et al. 1992). The third phase at the Miocene/Pliocene boundary coincided with a global cooling trend, the spread of grasslands, and the evolution of savannah-adapted bovids (Cerling et al. 1997)—all divergences that are all broadly consistent with estimates based on the cytochrome b sequences analyzed in the present study (Fig. 2). In summary therefore, pulses of divergence within Bovidae appear to coincide with times of climatic and vegetational change (Gentry 2000; Vrba and Schaller 2000; Fernández and Vrba 2005).

Fragmentation of habitat and concomitant small population size has been mooted as a major driver of genetic modification within bovids (Vrba and DeGusta 2004; Willows-Munro et al. 2005) providing as it does conditions that are intuitively conducive to fixing de novo chromosomal rearrangements through inbreeding and drift (White 1973, but see Coyne and Orr 2004 for a counter view), processes that may be facilitated by social structure (dominance hierarchies) in species (Bush et al. 1977) and demographic fluctuations. These would act to radically decrease effective population size. In fact, a recent survey of Late Neogene African fossils demonstrated that larger mammal species evolved from relatively small populations that subsequently increased rapidly in number and geographical spread (Vrba and DeGusta 2004). If small population size is central to chromosomal evolution in Bovidae, it is precisely at this point (small, probably initially allopatric populations) that the confounding effects of introgression could act to obscure phylogenetic relationships among future reproductively isolated species.

Interspecific hybridization is not uncommon in bovids and has, in fact, been suggested as a reason for the phylogenetic discordance between the mtDNA and nuclear genomes of the banteng species (Hassanin and Ropiquet 2007; Ropiquet et al. 2008). Outside of this and other examples within Bovini (e.g., Bos banteng × B. frontalis, B. banteng × B. indicus and B. taurus, B. bison × B. indicus, and B. taurus), documented cases of hybridization include species within Tragelaphini, Hippotragini, Cephalophini, Caprini, and Alcelaphini (Gray 1972; Van Gelder 1977; Robinson and Harley 1995; Ropiquet and Hassanin 2006). Hybridization followed by idiosyncratic lineage sorting of introgressed chromosomal mutations may result in phylogenetic discordance. Hemiplasy could similarly muddle relationships. If a chromosomal polymorphism persists across two or more successive nodes in a species phylogeny, it may eventually sort to fixation in ways that make the gene tree genuinely discordant with the topology of the species tree (Avise and Robinson 2008).

**Hemiplasy in Caprini, Bovini, Tragelaphini, and Reduncini**

There is a very real distinction to be made between the convergence or reversal of chromosomal rearrangements (true homoplasies) that develop through the propensity of acrocentrics to fuse/fission in bovids, and the homoplasy-like consequences resulting from the retention of polymorphisms through species divergences (hemiplasy; see Avise and Robinson 2008). Convergence of a chromosomal character that is promoted by nonallelic homologous recombination at meiosis, for example, would be likely to occur independently of the tight evolutionary time constraints required for hemiplasy (which requires short interval times). The probability for de novo rearrangements to include convergent
chromosomal changes would increase the greater the time post speciation. Put differently, chromosomal rearrangements are considered rare (the Rare Genomic Changes of Rokas and Holland 2000), and convergent rearrangements are unlikely when the divergence times leading to terminal taxa are short.

In contrast, one would anticipate that hemiplasy would more readily result where the internodes are short, and the persistence time of a polymorphism is relatively protracted. Persistence is more likely in instances where the rearrangement is overdominant (Stefansson et al. 2005) or where it is mildly underdominant (suspected with Rb fusions in cattle; Schmutz et al. 1996) but trapped by physical barriers to gene flow. Such a situation would permit rearrangements to remain polymorphic at the scale of the metapopulation for considerably long periods (Barton 1979; Barton and Hewitt 1985). In the case of overdominant rearrangements, for example, selection may be acting on underlying genetic variants associated with a particular rearrangement rather than the rearrangement itself (see, e.g., Faria and Navarro 2010 and references therein). Clearly, however, these would have to be tightly linked to the rearrangement, so that the probability of recombination removing the variants is reduced. Larkin et al. (2009) showed using multispecies genome comparisons that cattle-specific evolutionary breakpoint regions (EBRs; the interval between syntenic blocks that result from rearrangement) often contained genes with functions related to ruminant-specific and adaptive phenotypes. This was taken to suggest that mammalian chromosomal rearrangements may be adaptive although, quite clearly, not all the EBRs identified in this study necessarily result from Rb fusions. Similarly, Banaszek et al. (2008) have proposed that persistence of Rb fusion polymorphisms in the common shrew, Sorex araneus, is due to selection for higher metabolic rates and that this may outweigh the expected negative effects of Rb heterozygosity on fertility in this species. When rearrangements are selectively neutral, however, persistence would necessitate very large effective population sizes. Because population size is likely to vary over time, this would radically affect the probability of hemiplasy occurring under conditions of neutrality.

Of the 41 potential homoplasies/hemiplasies identified in our study, the analysis of character states in the terminal taxa using Mesquite suggests that six are hemiplastic (~15%) based on the relative likelihood pie diagrams and ancestral state reconstruction summaries (Fig. 3). In these reconstructions, state assignments were done using binary characters—specifically, the presence/absence of unambiguous rearrangements—and persistence times that were considered biologically realistic (i.e., when they occurred over relatively short evolutionary time scales). This was defined by the ~3 myr suggested for an inversion polymorphism under positive selection (see Stefansson et al. 2005), with the upper bound set by the 5–8 myr estimated for the lineage sorting of polymorphic sites in autosomal genes within Bos and Bison species (MacEachern et al. 2009).

Interestingly, the two subspecies of B. javanicus that have different chromosomal rearrangement (online Appendix 1, doi:10.5061/dryad.8332) similarly provide an opportunity to determine persistence times in this clade. Thus, if the assumption that hemiplasy is correct for this topology, then the Rb 2;28 fusion must have been polymorphic in the common ancestor to B. frontalis, B. j. javanicus, and B. j. birmanicus 2.57 Ma, and this was retained for at least 0.23 myr (Fig. 3). The hemiplastic characters identified in our study include two in Tragelaphini (character 117 = Rb 11:20 and character 135 = Rb 7:28), two in the Reduncini (character 91 = Rb 5:13 and character 123 = Rb 6:18), and one each in Caprini (character 71 = Rb 5:11) and Bovina (character 76 = Rb 2:28). The minimum persistence times for the six potentially hemiplastic characters are, with the exception of those in Tragelaphini, <3.2 Ma (0.23–3.23). The two characters in Tragelaphini would need to endure as polymorphisms for ~4.8 myr.

To explain the reasoning underpinning the genealogical discordance that could result from the existence of more than one allele (in this case alternate forms of a karyotypic feature) at an ancestral node (i.e., hemiplasy), we use the Reduncini as illustrative taxon. Similar reasoning can be applied to the karyotypic states identified in Caprini, Tragelaphini, and Bovina.

Close scrutiny of the chromosomal rearrangements defining species’ karyotypes within Reduncini (Table 1 and online Appendix 1, doi:10.5061/dryad.8332) shows that of the 10 Rb fusions, 7 are homoplasic (BTA3;11, BTA3;10, BTA5;17, BTA6;10, BTA4;7, BTA2;25; and BTA6;29) in that the same brachial combinations are also present in species of other bovid tribes, one (BTA1;19) defines the monophyly of Kobus, and two meet our criteria for hemiplasy. These are BTA5;13 (present in K. kob and K. leche) and BTA6;18 (present in K. ellipsiprymnus, K. leche, and K. megaceros; Fig. 3 and Table 1). Of these it is instructive to examine the BTA 6:18 fusion (character 123, Fig. 3), although equally applicable to BTA5;13 (Fig. 3). It could be argued that Rb 6:18 was fixed at the base of Kobus (6.28 Ma), and its absence in K. kob is due to a reversal or, alternatively, it was fixed in the common ancestor to K. leche and K. megaceros at 3.05 Ma, and convergently so in K. ellipsiprymnus. Two “rare genomic” changes would be required in either scenario. In contrast, hemiplasy would suggest a single fusion at the common node (6.28 Ma) followed by incomplete lineage sorting when the ancestral polymorphism is retained through speciation events. This requires a persistence time of 3.23 Myr and a single “rare genomic” change.

In interpreting these patterns, however, it should be noted that introgression can phylogenetically mimic both homoplasy and hemiplasy because it too violates the assumption of evolution from a common ancestor through a bifurcating process. This raises the possibility that the phylogenetic conflict evidenced by chromosomal characters may reflect past bouts of introgression. All the homoplases identified in the Reduncini (i.e., BTA3;11, BTA3;10, BTA5;17, BTA6;10;
BTA4;7, BTA2;25, and BTA6;29) transcend tribal affiliations (i.e., are ancient; online Appendices 1 and 2, doi:10.5061/dryad.8332) and to account for this, hybridization and subsequent lineage sorting must have occurred contemporaneously with radiation of extant tribes (deeper portions of the species tree). The persistent times necessary for lineage sorting to accommodate the observed patterns are improbable, and these are therefore instances of true homoplasy. Nonetheless, our arguments that BTA5;13 (present in K. kob and K. leche), BTA6;18 (present in K. ellipsiprymnus, K. leche, and K. megaceros) reflect instances of hemiplasy must be tempered by recorded instances of hybridization among many species of bovids and, particularly pertinent to the present study, within Kobus (Gray 1972).

Introgressive hybridization would, however, necessitate a very restrictive set of circumstances. This would require the independent fixation of a Rb fusion in a reproductively nonisolated species, the production of fertile hybrids between it and another reproductively nonisolated species, the spread and fixation of the introgressed rearrangement, and a subsequent speciation phase that would result in gene-tree/species-tree discordance. Consequently, although both hemiplasy and introgression can lead to complications in chromosomal phylogenies, it seems more plausible to consider that, under the constraints above, some of the “convergent” Rb fusions in the Reduncini phylogeny most likely reflect instances of genuine trait homology and shared ancestry, and thus hemiplasy.

In conclusion, although progress has been made in examining the effects of hybridization (Mallet 2005, 2007; Meng and Kubatko 2009), horizontal gene transfer (Beiko et al. 2008) and incomplete lineage sorting (Degnan and Salter 2005; Degnan and Rosenberg 2009) in molecular phylogenetics, the present investigation represents a first attempt to grapple with the complexities introduced by hybridization and coalescences of lineages using chromosomal data. We show that the dominance of Rb fusions and the tendency for this class of rearrangement to arise convergently confounds the retrieval of an accurate history of bovid phylogenetic relationships. In future, however, examining sources of genealogical discordance may benefit from detailed analysis of the X chromosome because high density BAC mapping and the use of microdissected painting probes, principally in domestic species (reviewed in Iannuzzi et al. 2009), has revealed intrachromosomal shuffling of this chromosome. If this variation proves extensive and holds in a taxonomically broader and species-rich sample, these rearrangements (which may be less prone to convergence) could, potentially, be better suited to resolving close species relationships and hence the phylogenetic ramifications of hemiplasy, homoplasy, and hybridization in this group of mammals.
SUPPLEMENTARY MATERIAL

Supplementary material, including data files and/or online-only appendices, can be found at http://dx.doi.org/10.5061/dryad.8332.

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