Supplemental Results

On UCEs as loci for phylogenetic inference

Ultra-conserved elements (UCEs) have proven increasingly popular and useful as loci for phylogenomic analyses across a range of taxonomic levels and time scales (Crawford et al. 2012; McCormack et al. 2012; Faircloth et al. 2013; McCormack et al. 2013; Jarvis et al. 2014; Smith et al. 2014; Blaimer et al. 2015; Meiklejohn et al. 2016; Alexander et al. 2017; Burress et al. 2018; Bossert et al. 2019; Oliveros et al. 2019; Quattrini et al. 2020; Alda et al. 2021; McLean et al. 2022). UCEs are typically collected using bait capture and targeted enrichment approaches, harvested from whole genomes as we have done, or a combination of the two. This is driven by both their ready application to non-model organisms and museum specimens (Bi et al. 2013; Faircloth 2017; Zhang et al. 2019), their possession of desirable characters for use in phylogenetic reconstruction (Faircloth et al. 2012; McCormack et al. 2012), and their subsequent ability to provide resolutions to previously intractable nodes (Faircloth et al. 2013; Blaimer et al. 2015; Alda et al. 2021). However, both the function of UCEs and the influence of selection upon them remain poorly understood (Bejerano et al. 2004; Katzman et al. 2007). Rates of molecular evolution may also vary within UCE loci (Tagliacollo and Lanfear 2018) and phylogenetic reconstructions using UCEs (and other phylogenomic markers) can be confounded by gene tree discordance driven by processes such as incomplete lineage sorting and introgression (Jeffroy et al. 2006; Degnan and Rosenberg 2009; Meiklejohn et al. 2016; Chan et al. 2020; Alda et al. 2021).

While UCEs are generally treated as non-coding and independent, they may not be evenly distributed throughout the genome (McCole et al. 2018) and multiple UCEs may overlap the same gene (Van Dam et al. 2021). Merging UCEs that overlap genes can increase topological support but has limited impact on the topology recovered (Van Dam et al. 2021). When available, filtering by phylogenetic signal and noise (Gilbert et al. 2018) can also lead to better supported estimates of tree topology, as can allele phasing of SNPs derived from UCE loci (Andermann et al. 2019; McLean et al. 2022). However, in assessing that support, we notice in our reconstructions and as has been observed elsewhere with multiple phylogenomic markers (Reddy et al. 2017; Chan et al. 2020; Minh et al. 2020; Vanderpool et al. 2020), bootstraps are a poor indicator of support for the correct topology when using phylogenomic data.

UCEs have recently seen increased use in dating analyses and best practices continue to be developed (Blaimer et al. 2015; Branstetter et al. 2017; Bossert et al. 2019; Oliveros et al. 2019; Quattrini et al. 2020). Critically, the scale of UCE datasets can make popular methods of divergence time estimation computationally intractable, while gene tree discordance and substitution rate heterogeneity complicate the selection of appropriate models (Van Dam et al. 2017; Tagliacollo and Lanfear 2018). One strategy to overcome these issues is the selection of a set of loci with model-appropriate properties including clock-like behavior and low gene tree discordance, as described by the SortaDate software package (Smith et al. 2018). Such gene filtering approaches have been suggested as best practice (Walker et al. 2019) and SortaDate

40 specifically has been used in a range of phylogenomic analyses across multiple taxonomic groups
41 (Lind et al. 2019; Del Cortona et al. 2020; Quattrini et al. 2020; Shee et al. 2020; Koenen et al.
42 2021), though some researchers have found gene filtering has a limited impact on estimated
43 divergence times but increases the associated variance (Oliveros et al. 2019; McGowen et al.
44 2020).

45   For dating strategies, least-squares purports vastly reduced computation time with similar
46 accuracy (To et al. 2016) to comparable dating methods implemented in software such as BEAST
47 (Drummond et al. 2006) and r8s (Sanderson 2003). It appears to be robust to topological error (To
48 et al. 2016) and substitution rate heterozygosity between lineages (To et al. 2016; Tong et al. 2018).
49 While developed for dating of rapidly evolving viruses (To et al. 2016), it has seen use in
50 eukaryotes (Yue et al. 2017; Anijalg et al. 2018; Brüniche–Olsen et al. 2018; Tong et al. 2018;
51 Thomas et al. 2019). Given the known rapid evolution of substitution rate in rodents (Douzery et
52 al. 2003), our concerns with tree topology, and its tractability, it is likely appropriate for our dataset
53 of filtered UCE loci.

54 *Divergence time discussion*

55 When estimating divergence times on our inferred species trees, the Eumuroidea root (A) is placed
56 at 22.66 Ma, which is concordant with the reconstruction of Schenk et al. (2013). The range
57 between minimum and maximum reconstructed age is wide, overlapping both the maximum first
58 appearance and estimated divergence time for the clade as described by Steppan and Schenk
59 (2017). The estimated date for Muridae (B) of 21.34 Ma likewise has a wide range, and appears to
60 be approximately 0.5 (Schenk et al. 2013), 5 (Chevret and Dobigny 2005), or 8 (Steppan and
61 Schenk 2017) million years older than other estimates. It aligns well with the estimated age of the
62 clade recovered by Aghová et al. (2018) and, with some variance for the dating method used, the
63 supermatrix derived mammalian tree of Meredith et al. (2011) but, as with other nodes, is much
64 younger than was estimated by Hedges et al. (2015). The time of separation of Otomyini and
65 Arvicanthi (Fig. 1, node N) is in general agreement with Lecompte et al. (2008), Schenk et al.
66 (2013), and Aghová et al. (2018) at 8.22 Ma. As with Steppan and Schenk (2017), we reconstruct
67 the origins of Arvicanthini (Fig. 1, node O, 6.56 Ma), Praomyini (node I, 4.83 Ma), and Murini
68 (node K, 6.25 Ma) as approximately 0.5-1 Ma younger than previously determined (Lecompte et
69 al. 2008; Schenk et al. 2013; Aghova 2018), though these older estimates overlap our
70 confidence intervals and Nicolas et al. (2021) have recently estimated an even earlier
71 diversification for Praomyini (7.1 Ma). Within the Murini (node K), our divergence of 3.36 Ma
72 for *M. caroli* (node L) followed by the separation of *M. musculus* and *M. spretus* (node O, 1.38
73 Ma) is very similar to estimates by Suzuki et al. (2004).

74 *Species tree summary*

75 Phylogenomic datasets represent a wealth of opportunity to better understand taxonomic
76 relationships, but their size and complexity can make them challenging to use and prone to
77 introducing error (Young & Gillung, 2020; Zhang et al, 2019). As our analyses are based on the
78 available whole genomes of murid rodents, we are inevitably limited in our taxon sampling.

79   Coalescent methods such as ASTRAL-III (Zhang et al., 2018) appear to be resilient to analyses
80   on a small number of taxa (Song et al., 2012; Xi et al., 2015), though adding taxa to a dataset is
81   well understood to improve phylogenetic resolution in general (see Bravo et al., 2019 and
82   citations therein for a discussion). In contrast, adding more genomic data rather than taxa can,
83   counterintuitively, lead to increased support for erroneous topologies (Kumar et al., 2012;
84   Roycroft et al., 2019). It remains to be seen therefore the degree to which the topologies we
85   recover are the result of taxon sampling versus underlying properties of the data, although the
86   extent of gene tree discordance we observe remains striking. Ultimately, choice of data and the
87   models used may matter more than taxon sampling (Reddy et al., 2017), though knowing which
88   data to choose a priori remains an unsolved problem.
89   Here, we have leveraged the resources of the model organisms the house mouse (*Mus*
90   *musculus*) and brown rat (*Rattus norvegicus*) along with new genomes from eight closely related
91   species and eight previously sequenced rodent genomes to understand the systematics of murine
92   rodents and causes and consequences of phylogenetic discordance along the murine genome. Our
93   genomes begin to fill the gap in sampling of murine rodents which, despite their outstanding
94   species diversity, have relatively few whole genomes sequenced and help to place these
95   important model systems in an evolutionary context and provide us with the resources to study
96   the landscape of phylogenetic discordance along the chromosome.

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Supplemental Figure legends

Figure S1: Species trees inferred from 2,632 UCE loci. A: Species tree as estimated from concatenation of all loci by IQ-TREE2, with branches showing SH-aLRT/UFBootstrap supports. B: ASTRAL-III species tree based on individual gene trees with internal branch lengths in coalescent units such that shorter branches indicate greater discordance. Branch labels indicate quadripartition support. Final normalized quartet score is 0.81, *i.e.*, 81% of quartet trees induced by the gene trees are represented in the species tree.

Figure S2: Gene-concordance factors (gCF) and site-concordance factors (sCF) for each branch in the concatenated species tree. Note that the lowest possible value for sCF is approximately 30, while gCF can be as low as 0.

Figure S3: Discordance at nodes on the ASTRAL species tree established using the PhyParts package (Smith et al., 2015). The number of gene trees that support the depicted species tree at each node is given above each branch and is represented by the blue portion of the pie chart. The number of gene trees that show a supported conflict with the gene tree is below the branch and is represented in pie charts by green (the most common conflicting partition) and red (all other supported conflicts). The grey section of the pie charts show conflicting gene trees with no support.

Figure S4: The number of variable and informative sites from 165,409 10kb windows from alignments of seven taxa to the mouse reference (mm10) coordinate system. A variable site is defined as any site with more than one allele. An informative site is defined as any site with at least two alleles present in at least two taxa. Average number of variable sites: 1187.8. Average number of informative sites: 401.0.

Figure S5: Measures of phylogenetic similarity and decay on the X chromosome. A) The log fit to the mean of distributions of tree distances between windows at increasing genomic distance (10kb steps). B) The same, but on a log scale with a linear fit. C) The genomic distance between windows at which tree distance becomes random for 100 replicates of random window selection. D) The slopes of the correlation between genomic distance and tree distance from panel B represent the rate at which tree similarity decays across the chromosome.

Figure S6: Dotplot of whole genome alignment between mouse (mm10) and rat (rnor6) genomes.

Figure S7: Summary of the whole genome alignment between mouse and rat. A) The distribution of aligned block sizes. B) The distribution of the total number of aligned bases for each range of block sizes. C) The distribution of inter-block distances relative to mouse (left) and rat (coordinates) for all blocks shorter than 20 kb. Dashed lines represent average distance between two alignment blocks and are labeled with that average.

Figure S8: Distributions of recombination rate across 19 mouse autosomes and the X chromosome estimated in 5Mb windows. Each line segment represents a single aligned block between the two genomes.

Figure S9: Various correlations between recombination rate, phylogenetic similarity, genomic features, and structural variation (from mouse to rat).

Supplemental Tables

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143 **Table S1: All taxa whose genomes were included in this study, the source of the assembly,**
144 **and the assembly level of each genome. For the six samples used in the genome-wide**
145 **discordance analyses (column 5, except for mm10), we also generated reference-based**
146 **pseudo-assemblies using the mouse genome (mm10) as the reference.**

Taxon	Assembly source	Assembly level	No. UCEs	Used in genome-wide discordance analyses
Apodemus speciosus	GenBank: GCA_002335545.1	Scaffolds	2336	
Apodemus sylvaticus	GenBank: GCA_001305905.1	Scaffolds	2510	
Arvicanthis niloticus	GenBank: GCA_011762505.1	Chromosomes	2563	
Grammomys dolichurus	Kumon et al., 2021: *de novo* assembled	Chromosomes	2395	*
Hylomyscus alleni	Kumon et al., 2021: *de novo* assembled	Chromosomes	2392	*
Mastomys natalensis	Kumon et al., 2021: *de novo* assembled	Chromosomes	2483	*
Mus caroli	GenBank: GCA_900094665.2	Chromosomes	2584	
Mus musculus	GenBank: GRCm38.p6/mm10	Chromosomes	2294	*
Mus pahari	GenBank: GCA_900095145.2	Chromosomes	2556	
Mus spretus	GenBank: GCA_001624865.1	Chromosomes	2578	
Otomys typus	This study: *de novo* assembled	Scaffolds	2627	
Phodopus sungorus	Moore et al., 2022: *de novo* assembled	Chromosomes	2633	
Praomys delectorum	Kumon et al., 2021: *de novo* assembled	Chromosomes	2549	*
Rattus norvegicus	GenBank: GCA_015227675.2	Chromosomes	2425	
Rattus rattus	GenBank: GCA_011064425.1	Chromosomes	2443	
Rhabdomys dilectus	Kumon et al., 2021: *de novo* assembled	Chromosomes	2546	*
Rhombomys opimus	GenBank: GCA_010120015.1	Scaffolds	2627	
Rhynchomys soricoides	Kumon et al., 2021: *de novo* assembled	Chromosomes	2570	*

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Table S2: The estimated age of each node in Figure 1. Nodes with an asterisk (*) indicate those used in calibration, as in Table 2.

Node	Estimated Age (mya)	Min/Max Age (mya)
A	22.67	17.75/28.66
B	21.34	16.77/26.01
C	13.11	11.42/15.10
D	12.15	11.10/13.51
E*	11.70	11.10/12.30
F	10.84	9.62/11.96
G*	5.86	4.57/6.96
H	10.20	8.81/11.41
I	4.83	3.69/5.88
J	4.46	3.34/5.33
K*	6.25	4.92/7.48
L	3.36	2.49/4.13
M	1.38	0.99/1.92
N*	8.22	6.74/9.20
O	6.57	5.21/7.82
P	4.34	3.28/5.44
Q	2.02	1.26/3.00

Table S3: Fossil calibrations used in molecular dating. Ages are provided in millions of years, and "NA" indicates that no minimum age was specified.

Fossil	Clade	Minimum Age	Maximum Age	Citation
Karnimata sp.	Core Murinae	11.1	12.3	Kimura et al. (2015); (Kimura et al. 2016)
Karnimata darwini	Otomyini + Arvicanthini	NA	9.2	Kimura et al. (2015)
Parapodemus lugdunensis	*Apodemus*	NA	9.6	Daxner-Höck (2002)
Mus sp.	*Mus*	NA	8.0	Kimura et al. (2013); (Kimura et al. 2015)

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