**The genomic landscape, causes, and consequences of extensive phylogenomic discordance in Old World mice and rats**

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# Abstract

The tree of life is a central concept in evolutionary biology. This tree is represented as a branching graph known as a phylogeny, which can show the entire history of a set of species. When reconstructing phylogenies from genomic data, we often combine markers from multiple regions of the genome to infer a single species tree. However, the history of the underlying loci can differ from the history of the inferred species tree. The causes of this discordance are well explored, but its effects on genome evolution and its impact on comparative genomics is under-appreciated. Here, we characterize patterns of phylogenomic discordance across the murine rodents—one of the most specious clades of mammals that has diversified rapidly in the past 15 Ma – and whose evolutionary history may have significant human health implications. Using new genome assemblies for 8 murine rodent species, as well as previously published data, we infer a robust evolutionary history of this globally distributed and ecologically diverse group from ultra-conserved regions of the genome, building on prior genomic efforts in this group which have concentrated on a few well-studied model systems like the house mouse and the brown rat. Though the species tree inferred from conserved elements is well supported, we observe high levels of discordance among individual loci. We expand our scope from these loci to examine discordance across the rodent genome and find that phylogenies built from physically proximate genomic regions have similar topologies, and that this similarity decays in consistent patterns, for example, near UCEs. We also show that using a single species tree can result in both false positives and false negatives when inferring genes under positive selection. We use our combined dataset to Our findings highlight the complexity of phylogenetic sampling with respect to gene-tree species-tree discordance, and we ultimately recommend that the locus-tree should be used over the species tree when investigating molecular evolutionary processes.

# Introduction

Phylogenies are the unifying concept in understanding the evolution of species, traits, and genes. However, as the revolution in high-throughput sequencing has expanded our knowledge of heterogenous patterns across the genome , it is no longer controversial to acknowledge that a single evolutionary history may not adequately represent the evolutionary relationships between species (Edwards 2009; Hahn and Nakhleh 2016). While a dominant signal of bifurcating speciation may exist (i.e., a species tree), persistence of ancestral polymorphisms leading to incomplete lineage sorting (ILS), gene flow through hybridization (introgression), and gene duplication and loss can generate patterns of phylogenetic discordance among trees at individual loci and inferred species trees (Maddison 1997). The theoretical prediction of phylogenetic discordance has been appreciated for more than three decades (Pamilo and Nei 1988; Maddison 1997; Rosenberg 2002; Avise and Robinson 2008), but empirical evidence is now driving home how much discordance can exist among a set of species. For instance, studies of birds (Jarvis et al. 2014), seals (Lopes et al. 2021), tomatoes (Pease et al. 2016), and insects (Sun et al. 2021) have found that even with extensive taxon sampling highly supported species trees are rarely or never recovered in the underlying gene-trees. While these examples highlight the prevalence of phylogenetic discordance across the tree of life, the availability of high-quality genome assemblies now provides an opportunity to examine how discordance varies as a function of basic genome biology and its consequences on inference of evolutionary processes in comparative studies that assume a singular species history.

From a practical perspective, failure to acknowledge and account for phylogenetic discordance could have severe consequences on biological inference. Analyses of molecular evolution are usually performed on a gene-by-gene basis (Pond et al. 2005; Yang 2007; Hu et al. 2019; Kowalczyk et al. 2019), but it is still common practice to assume a single genome-wide species tree. For gene-based analyses, using the wrong tree may compromise inferences of positive directional selection, convergent evolution (Mendes et al. 2016), or genome-wide inferences of correlated rate variation (Mendes et al. 2016). Phylogenetic discordance can also affect how continuous traits are reconstructed across phylogenies, as the genes that underly these traits may not follow the species history (Hahn and Nakhleh 2016; Mendes et al. 2018). In these instances, phylogenetic discordance needs to be characterized and incorporated into the experimental and analytical design. Alternatively, if a researcher’s primary questions are focused on reconstructing the evolutionary history of speciation (i.e., the species tree), then phylogenetic discordance can mask the true signal of speciation. In this case, efforts to sample genetic variation from genomic regions less prone to discordance might be preferable.

Given these considerations, a better understanding of the genomic context of phylogenetic discordance is warranted. Although often conceptualized as a stochastic consequence of population history, patterns of phylogenetic discordance are likely to be non-random and structured across the genome, dependent on localized patterns of genetic drift, natural selection, recombination, and mutation. Discordance due to ILS ultimately depends on effective population size (Pamilo and Nei 1988; Degnan and Rosenberg 2006), and therefore should covary with any process that influences local patterns of genetic diversity (e.g., linked negative or positive selection). Likewise, the potential for discordance due to introgression may be influenced by selection against incompatible alleles or, less often, positive selection for beneficial variants (Lewontin and Birch 1966; Jones et al. 2018). Differences in how these potential sources of discordance arise allow us to test hypotheses about both the cause and the scale of phylogenetic discordance across the genome (Huson et al. 2005; Kulathinal et al. 2009; Green et al. 2010; Vanderpool et al. 2020). However, the genomic context of phylogenetic discordance has remained elusive. For example, the potential for any of these processes to generate localized patterns of phylogenetic discordance depends on patterns recombination because linkage disequilibrium is lost over time, breaking up genomic variation that shares histories (Hudson and Kaplan 1988). Prior empirical studies have been inconclusive regarding the relationship between phylogenetic discordance and recombination rate—for example, great apes show no correlation (Hobolth et al. 2007) and several mouse species have a significant but weak correlation (White et al. 2009). Nonetheless, more recent simulation studies posit that phylogenies in the genome are expected to be correlated – the closer two regions are in the genome, the more history they share (McKenzie and Eaton 2020). However, if recombination rates evolve sufficiently quickly, then long-term discordance measured over evolutionary timescales might be largely independent of contemporary recombination landscapes. Lanier and Knowles (2012) show that recombination does not affect species tree inference while the amount of discordance from ILS does, suggesting that the relationship these two phenomena may not be detectable at evolutionary timescales.

To investigate the causes and consequences of phylogenetic discordance, we took advantage of genomic resources available for house mouse (*Mus musuculus*) and brown rat (*Rattus norvegicus*). These rodent species are the two most important mammalian model systems for biological and biomedical research, and they are embedded within a massive radiation of Old-World rats and mice (Murinae). This ecologically diverse and species rich group is comprised of over 600 species and makes up >10% of all mammalian species, and yet is only about only ~15 million years old, and yet. Other than *Mus* and *Rattus*, few other murine genomes have been generated; most efforts have focused on sampling variation within a few lineages of house mice. Previous efforts to quantify discordance in rodent species have found it is widespread across the genome, but these comparisons have been limited to a few *Mus* species (White et al. 2009).

In the present work, we report genome sequences of seven new murine rodent genomes (*Mastomys natalensis*, *Hylomyscus alleni*, *Praomys delectorum*, *Rhabdomys dilectus*, *Grammoyms dolichurus*, *Otomoys typus*, and *Rhynchomys soricoides*) sampled from across this radiation. We combine these genomes with previously sequenced rodent genomes and genomic resources from the *M. musculus* model system to study the phylogenetic relationships within Murinae as well as the landscape of discordance along rodent chromosomes. We first inferred a species tree for these and other sequenced rodent genomes, focusing on signal derived from ultra-conserved elements (UCEs) to promote broader comparisons. Driven by their conserved phylogenetic signal which helps to resolve difficult relationships (Faircloth et al. 2013; Blaimer et al. 2015; Alda et al. 2021), UCEs have proven increasingly popular and useful as loci for phylogenomic analyses across a range of taxonomic levels and time-scales (e.g. (Blaimer et al. 2015; Alexander et al. 2017; Quattrini et al. 2020; Alda et al. 2021) (Salter et al. 2022). We then use whole genome sequences, genetic maps, and annotation information from *Mus musculus* to describe the genomic context of phylogenetic discordance at a broader taxonomic scale and evaluate several hypotheses linking discordance to by genetic drift, natural selection, and recombination. Finally, we show how the use of a species-tree over a gene-tree impacts common molecular evolution tests for natural selection in these species.

# Methods

## Sample collection

(from Penn group)

## Sequencing and assembly

(from Penn group)

Genome assembly sources are identified in Table 1. Library prep information will be provided by Tomo. We sequenced genomes from eight murine species for this study using the 10x Genomics sequencing platform and – except for *Otomys typus* – assembled to the mm10 reference using LongRanger v2.2.2 (Marks et al. 2019).

As the library quality for *O. typus* was too low for chromosome level assembly, we instead assembled it into scaffolds with the express purpose of obtaining UCEs. Adapters and low-quality bases were trimmed from the reads using illumiprocessor (Faircloth 2013), which makes use of functions from trimmomatic (Bolger et al. 2014). All cleaned reads were de novo assembled using ABySS 2.3.1 (Jackman et al. 2017) with a Bloom filter (Bloom 1970) de Bruijn graph. The final *O. typus* scaffold assembly was 2.14GB (N50=9,211; L50=64,014; E-size=12,790).

## Ultraconserved element (UCE) retrieval

With the goal of reconstructing a broad phylogeny of murine rodents, we combined our seven newly sequenced genomes with nine publicly available genomes from other Old World mice and rats (subfamily: Murinae) as well as the genomes of two non-murine rodents, the great gerbil (*Rhombomys opimus*) and the Siberian hamster (*Phodopus sungorus*) as outgroups. We extracted UCEs from each species using the protocols for harvesting loci from genomes and the *Mus musculus* UCE probe set provided with PHYLUCE (Faircloth et al. 2012; Faircloth 2016). For our new genome assemblies based on the *Mus musculus* reference genome (see Table 1), we matched the coordinates of UCEs from the reference mouse genome using the liftOver tool in the UCSC Genome Browser (Hinrichs et al. 2006) and extracted these sequences along with 1500 flanking base pairs on both ends of the element. We harvested UCEs for the remaining using the standard PHYLUCE protocol (Faircloth 2016). In total, we recovered 2,645 unique UCE loci, though not all UCE loci were found in all taxa. The *Rattus* species yielded fewer UCE loci, as did the scaffold level assemblies from GenBank (Table 1).

## UCE alignment

We brought the extracted UCE sequences for each species into a consistent orientation using MAFFT v7 (Katoh and Standley 2013) and then aligned them using FSA (Bradley et al. 2009) with the default settings. We trimmed UCE alignments with TrimAl (Capella-Gutierrez et al. 2009), concatenated them, and performed additional quality checks using Sequence Matrix v1.8 (Vaidya et al. 2011) and AMAS (Borowiec 2016) to find the optimal filtering parameters (50% occupancy). All alignments were processed in parallel with GNU Parallel (Tange 2018).

## Species tree reconstruction from UCEs

We constructed a species level rodent phylogeny with two approaches. First, using the concatenated alignments of all UCEs, we reconstructed a maximum-likelihood (ML) species tree with IQ-TREE 2 (Minh et al. 2020b) and its inbuilt partition model (Chernomor et al. 2016). Next, we used IQ-TREE 2 to reconstruct gene-trees from the individual UCE alignments. We set IQ-TREE to infer an optimal substitution model for each alignment with ModelFinder (Kalyaanamoorthy et al. 2017). For all IQ-TREE runs (concatenated, or individual loci), we assessed branch support with ultrafast bootstrap approximation (UFBoot) (Hoang et al. 2018) and the corrected approximate likelihood ratio test (SH-aLRT) (Guindon et al. 2010). We collapsed branches in each UCE tree exhibiting less than 10% bootstrap support using the nw\_ed function from Newick Utilities (Junier and Zdobnov 2010) and used these trees as input to the quartet summary method ASTRAL-III v5.15.3 (Zhang et al. 2018) to infer a species tree. We generated visualizations of phylogenies with R v4.1.1 (R Core Team 2021) using phytools (Revell 2012) and the ggtree package v3.14 (Yu et al. 2017; Yu 2020) and its imported functions from ape v5.0 (Paradis and Schliep 2019) and treeio v1.16.2 (Wang et al. 2020).

*Estimating patterns of discordance between UCE and species trees*

Phylogenetic discordance occurs when branching patterns between pairs of gene trees and between a gene tree and the species tree differ. We used two methods of assessing discordance across the reconstructed species tree. First, we calculated site- and gene- concordance factors (sCF and gCF) with IQ-TREE 2 (Minh et al. 2020a; Minh et al. 2020b) to assess levels of phylogenetic discordance between the inferred UCE trees and the concatenated species tree. gCF is calculated for each branch in the species tree as the proportion of UCE trees in which that branch also appears (Baum 2007). sCF represents the proportion of alignment sites that are concordant with a given species tree branch in a randomized subset of quartets of taxa (Minh et al. 2020a). We visualized gCF and sCF for each branch in each species tree using methods in R v4.1.1 (Lanfear 2018; R Core Team 2021). Next, we used PhyParts (Smith et al. 2015b) to identify topological conflict between the UCE trees and the species tree from ASTRAL-III. For this analysis, we rooted all trees with *Phodopus sungorus* as the outgroup using the nw\_reroot function in the Newick Utilities (Junier and Zdobnov 2010) package and excluded 204 UCE trees that did not contain the outgroup. We visualized the PhyParts (Smith et al. 2015b) output with code from Matt Johnson (Johnson 2017), which utilizes Python 2.7 and the ETE toolkit v3.0.0b34 (Huerta-Cepas et al. 2016).

## Divergence time estimation

We used IQ-TREE 2’s (Minh et al. 2020b) implementation of least square dating to estimate branch lengths of our species trees in units of absolute time (To et al. 2016). To improve divergence-time estimation, we used SortaDate (Smith et al. 2018) to identify a set of 10 UCE loci that exhibit highly clocklike behavior and minimized topological conflict with the concatenated species tree. We applied node age calibrations (Table 2) from Schenk et al. (2013) and Steppan and Schenk (2017), which in turn were sourced from fossil calibrations described on Paleobiology Database (2011). As *Rattus* is paraphyletic, the maximum age is taken from the earliest crown group fossil on Paleobiology Database (2011) while the estimated *Rattus* node age from Schenk et al (2013) was used as the minimum age. Branch lengths were resampled 100 times to produce confidence intervals. To return a single solution, least square dating typically requires that one calibration be fixed, and not a range. We selected one calibration node (here, the branch leading to Murinae) and estimated dates across the tree when this node is set to its minimum, its maximum, and its midpoint ages. On the midpoint calibrated tree, we plot confidence intervals for each node representing the lowest minimum and highest maximum ages that were estimated across the three dating analyses.

## Window-based analysis of phylogenies

To assess the distribution of phylogenetic discordance across the rodent genome, we limited subsequent analyses to six of the newly sequenced genomes (*Mastomys natalensis*, *Hylomyscus alleni*, *Praomys delectorum*, *Rhabdomys dilectus*, *Grammomys dolichurus*, and *Rhynchomys soricoides*). *Otomys typus* was excluded from these analyses due to the library quality problems outlined above. For these genomes we generated consensus sequences with bcftools (Danecek et al. 2021) using the reads mapped to the *M. musculus* reference genome to retain collinearity between genomes. In detail, we removed SNPs that did not pass LongRanger’s quality filters (bcftools view -f PASS), removed duplicate SNPs, and normalized indels (bcftools norm -c e -d all). We also corrected the AN and AC fields in each VCF file (bcftools plugin fill-AN-AC). Finally, we filtered indels closer than 5bp such that only the longest one was retained (bcftools filter –IndelGap 5) and the consensus sequence was generated (bcftools consensus).

Next, we partitioned the genomes into 10 kilobase (kb) windows based on the coordinates in the reference *Mus musculus* genome (mm10; (Mouse Genome Sequencing et al. 2002)). These coordinates were converted between the reference and the consensus sequence for each genome using liftOver (Hinrichs et al. 2006). Note that this method assumes both collinearity of all genomes and similar karyotypes (see Discussion). We then removed windows from the subsequent analyses if (1) 50% or more of the window overlapped with repeat regions from the *M. musculus* reference RepeatMasker (Smit et al. 2013-2015) file downloaded from the UCSC Genome Browser’s table browser (Hinrichs et al. 2006) or (2) 50% or more of the window contained missing data in 3 or more samples. Overlaps with repeat regions were determined with bedtools coverage (Quinlan and Hall 2010). We then aligned the 10kb windows with MAFFT (Katoh and Standley 2013) and inferred phylogenies for each with IQ-TREE 2 (Minh et al. 2020b) which uses ModelFinder to determine the best substitution model for each window (Kalyaanamoorthy et al. 2017). To assess patterns of tree similarity between windows on the same chromosome, we used the weighted Robinson-Foulds (wRF) (Robinson and Foulds 1981; Böcker et al. 2013) distance measure implemented in the phangorn library (Schliep 2011) in R (R Core Team 2021). This measure compares two trees by finding clades or splits present in one tree but not the other. The weighted version of this measure increments a score by the missing branch length in each tree for each mismatch. We compared wRF between trees from windows on the same chromosome to characterize (1) heterogeneity in patterns discordance along the chromosome and (2) whether tree similarity is correlated with distance between windows. For the second question, we sampled every window on a chromosome at increasing distance (in 10kb windows) until the distribution of wRF scores for all pairs of windows at that distance was not significantly different (Wilcox test, p > 0.01) than that of a sample of 12,000 measures of wRF between randomly selected trees on different chromosomes. We selected 12,000 as the random sample size because it roughly matched the number of windows on the largest chromosome (chromosome 1, n = 12,113).

## Recombination rates

We retrieved 10,205 genetic markers generated from a large heterogenous stock of outbred mice (Shifman et al. 2006; Cox et al. 2009) to assess how phylogenetic discordance along chromosomes may or may not correlate with recombination rate. We converted the physical coordinates of these markers from build 37 (mm9) to build 38 (mm10) of the *M. musculus* genome using liftOver (Hinrichs et al. 2006). We then partitioned the markers into 5Mb windows and estimated local recombination rates in each window. Estimated recombination rates were defined as the slope of the correlation between the location on the genetic map and the location on the physical map of the *M. musculus* genome for all markers in the window (White et al. 2009; Kartje et al. 2020). Within each 5Mb window, we calculated wRF distances between the first 10kb window and every other 10kb window.

*Genomic features*

We also compared the chromosome-wide wRF distances to those based on phylogenies from regions around several types of adjacent to genomic features. We retrieved coordinates from 55,297 *M. musculus* genes from Ensembl (release 99), all 3,129 UCEs from the *M. musculus* UCE probe set provided with PHYLUCE (Faircloth et al. 2012; Faircloth 2016), and 9,865 recombination hotspots from Smagulova et al. (2011). The recombination hotspot coordinates were converted between build 37 and build 38 using the liftOver tool (Hinrichs et al. 2006). For each feature, the starting window was the 10kb window containing the feature’s midpoint coordinate. We then calculated wRF between this window and all windows within 1Mb in either direction.

## Selection analysis

To test how tree misspecification affects analyses of selection, we retrieved 21,733 orthologous coding sequences between *M. musculus* and the six newly sequenced genome (Fig. 1; Table 1) Coding coordinates from *M. musculus* were transposed to the new assemblies via liftOver (Hinrichs et al. 2006) to account for indels and sequences retrieved with samtools faidx (Danecek et al. 2021). We used MACSE to align these coding regions, which can account for possible frameshifts and stop codons (Ranwez et al. 2018). In detail, before alignment we trimmed non-homologous regions from each ortholog using the trimNonHomologousFragments sub-program within MACSE. Then we aligned the orthologs using alignSequences and trimmed the aligned sequences with trimAlignment to remove unaligned flanking regions. After this, as a further alignment quality check, we manually filtered all alignments. We found that 3,214 alignments have a premature stop codon in at least one species, 1,758 alignments have 4 or more species with identical sequences, and 62 alignments are shorter than 100bp. We removed these alignments from all subsequent analyses, resulting in 16,733 total alignments for tree reconstruction and inference of selection.

We then used IQ-TREE 2 (Minh et al. 2020b) to reconstruct a single species tree from concatenation of all gene alignments, as well as gene-trees for each individual alignment. This species tree from coding regions matches the topologies of these species inferred by concatenation of UCEs in the previous section. Next we ran several tests that use both coding alignments and a tree to infer positive selection: PAML’s M1a vs. M2a test (Yang 2007), HyPhy’s aBSREL model (Smith et al. 2015a), and HyPhy’s BUSTED model (Murrell et al. 2015). We ran each test twice on each gene, once using the species tree derived from concatenated data, and once using the gene tree inferred from the alignment of that gene only. For the HyPhy models, no target branch was selected, meaning all branches in the input phylogeny were tested.

The end point of each of each of these three tests is a p-value, which allows us to assess whether a model that allows for positively selected sites fits better than a model that does not. For M1a vs. M2a, we obtained the p-value manually by first performing a likelihood ratio test to determine genes under selection by calculating . The p-value of this likelihood ratio is then retrieved from a one-tailed chi-square distribution with 2 degrees of freedom (Yang 2007). For BUSTED and aBSREL, p-values are computed automatically during the test using similar likelihood ratios. For the M1a vs. M2a and BUSTED tests, a single p-value is computed for each gene. P-values were adjusted by correcting for false discovery rates (Benjamini and Hochberg 1995; Yekutieli and Benjamini 1999) using the “fdr” method in the p.adjust() function in R (R Core Team 2021) and we categorized a gene as being positively selected if it’s adjusted p-value was < 0.01. For the aBSREL test, a p-value is generated for each branch in the input gene tree. aBSREL corrects for multiple testing internally across branches using the Holm-Bonferroni procedure (Holm 1979; Pond et al. 2005). We further correct the p-values across genes with the Bonferroni method and classify a gene as having experienced positive selection if one or more branches has a p-value < 0.01 after all corrections.

# Results

## Murine species tree

We sequenced and assembled genomes from seven murine species that represent some of the first non-*Mus* or *Rattus* genomes to be sequenced from the large radiation of Old World mice and rats. We combined our new genomes with nine publicly available murine genomes as well as the giant gerbil, *Rhombomys opimus* (Nilsson et al. 2020) and the Siberian dwarf hamster, *Phodopus sungorus* (Moore et al. 2022).We then inferred a species level phylogeny from ultra-conserved elements (UCEs). Using a concatenated dataset of 2,645 aligned UCEs we inferred a species tree (Fig. 1) that recovered the anticipated relationships between most murid taxa ((Steppan and Schenk 2017); Fig. S1) but placed the two *Apodemus* species and *Rhynchomys soricoides* in unexpected phylogenetic positions. With *Phodopus sungorus* separating the Cricetidae from the rest of Muridae, the first split was between the sole member of the Gerbillinae, *Rhombomys opimus*, and the Murinae. Within Murinae, we observed a deep split between the *Rattus* group and other taxa, in line with previous phylogenetic reconstructions of Murinae (Steppan et al. 2004; Lecompte et al. 2008; Steppan and Schenk 2017).

Based on previously inferred phylogenies from up to 6 loci, we would expect *Rhynchomys soricoides* to diverge next as the representative of Hydromyini, followed by a split with one lineage representing the African Otomyini and Arvicanthini, and another containing Murini, Apodemini, and Praomyini (Lecompte et al. 2008; Steppan and Schenk 2017). Instead, we found that the African group was rendered paraphyletic by *Rhynchomys* and *Apodemus*, while the expected relationships between Murini and Praomyini were recovered. The positions of *Apodemus* and *Rhynchomys* differ substantially from previous works (Steppan et al. 2004; Lecompte et al. 2008; Schenk et al. 2013; Steppan and Schenk 2017), even as bootstrapping and sh-aLRT values indicate high support for this topology overall. We took several approaches to assess the possibility of data or technical error in the phylogenetic pipeline (see Supplemental Results). Using individual UCE trees in combination with a multi-species coalescent-based inference method we recovered almost the same topology as the concatenated tree – including the unusual positioning of *Apodemus* (Fig. SXX). However, this tree has *Rhynchomys soricoides* separating after *Rhabdomys dilectus* and *Grammomys dolichurus*.

While bootstrap and SH-aLRT values provided high support to our inferred species trees (Fig. 1A), we found evidence for considerable discordance across individual UCE phylogenies. Just under half of the branches in the concatenated tree had a site concordance factor (sCF) of less than 50%, suggesting that alternate resolutions of the quartet had equivocal support (Fig. S2). Gene concordance factors (gCF) for each branch in the species tree were on aggregate much higher, with three branches supported by almost every gene tree in the analysis and with the lowest values likely being driven by a several short internal branches (Fig. S2). The deeper branches in the phylogeny showed less discordance, but some more recent nodes conflicted with between 50% and 75% of gene trees (Fig. XX). The branch leading to *R. soricoides* indicates particularly high conflict, but some discordance between the species tree and gene trees was found for all reconstructed relationships.

We estimated divergence times for the inferred concatenated phylogeny (Fig. 1; Table S2) using four fossil calibration points (Table 2). The murid and cricetid groups had an estimated divergence time of 24.27 Ma (node A in Fig. 1) followed by the Murinae and the Gerbillinae at 22.33 Ma (B), albeit with wide confidence intervals. With the ancestral Murinae node (C) fixed for calibration, the Otomyini and Arvicanthini arose at 11.90 Ma (D) and were followed by rapid diversification of the remaining Murinae (G, H, and J), with the Murini and Praomyini separating at 7.39 Ma (J). We also estimated a time-tree while filtering out species with elevated discordance and find similar key divergence times, though nodes closer to the root are marginally older, while nodes closer to the tips are very slightly younger on average (Supplemental Results; Fig. S3). Two nodes with fossil calibrations (the *Rattus* and *Apodemus* nodes) are recovered at the very youngest end of the calibration range.

## The landscape of phylogenetic discordance along rodent genomes

Limiting the number and nature of the loci used to resolve species relationships is important to get a clear picture of the species history, however, such targeted approaches are also likely to fail to capture the degree and genomic landscape of discordance. To describe this landscape in murines and test basic hypotheses relating to the effects of phylogenetic discordance, we next analyzed genome-wide phylogenetic histories of six newly sequenced murine rodent genomes (excluding *Otomys typis* due to low assembly quality) and the well-annotated *M. musculus* reference genome. Using the coordinate system from the *M. musculus* genome we partitioned and aligned 263,389 non-overlapping 10 kb windows from these seven species (Table 1).

After we filtered windows in repetitive regions or with low phylogenetically informative signal we recovered 164,198 phylogenies with an average of 401 informative sites per window (Fig. S4). We found that phylogenetic discordance was pervasive within and between chromosomes. We recovered 825 of the 945 possible unique rooted topologies among 6 species (when specifying *R. soricoides* as the outgroup) across all chromosomes (Table 3 shows the most common topologies recovered). The number of unique topologies per chromosome ranged from 160 to 388 with an average of 279 (Table 4). We ranked the recovered topologies by count per chromosome and found that eight different topologies were ranked in the top three in at least one chromosome. (Fig 2A; Table 3). The most frequent topology recovered across chromosomes matched the topology recovered via concatenation of all coding regions and, separately, that of the species tree recovered from UCEs (Fig. 1). However, this topology was only inferred for 12.5% of windows. That is, the robustly and consistently inferred species tree did not capture the evolutionary relationships inferred for nearly 90% of the genome. Among the top topologies, the relationships of *Hylomyscus alleni, Mastomys natalensis,* and *Praomys delectorum* (HMP clade) were discordant between the top two trees. In the third topology, the HMP clade matched the species topology but *Mus musculus* was no longer sister to it (Fig. 3B).

While relationships among windows varied greatly and visual inspection revealed no clear patterns of topological structure along the chromosome (e.g. Fig. 3C), we found that phylogenies were not randomly distributed across chromosomes. We measured tree distance between adjacent windows and compared these distances to those measured between randomly selected windows on different chromosomes. We found that tree similarity between windows decayed logarithmically along chromosomes (Fig. 3A and B) and the distance at which tree similarity appeared random varied among chromosomes from 0.8 Megabases (Mb) on chromosome 7 to 162.99 Mb on the X chromosome (Fig. 3C, Fig S5). While chromosomes 5, 10, and 11 were autosomal outliers with distances between windows to random-like trees exceeding 35 Mb, the average distance among all other autosomes was only 3.98Mb. The rates at which phylogenetic similarity decayed tended to be inversely proportional to the distance at which two randomly drawn phylogenies lost similarity (Fig. 3D).

## Discordance with recombination rate and other genomic features

Using markers from genetic crosses within *Mus musculus* (Shifman et al. 2006; Cox et al.) we examined whether regions of the genome with high recombination also showed more phylogenetic discordance over short genetic distances than regions with low recombination. Specifically, for each 5 Mb window we calculated the relationship between map location and the physical location for all markers within the window. Then within each 5 Mb window for which we had calculated recombination rate (Fig. S6), we measured tree similarity between the first and last 10 kb window. Surprisingly, we found no relationship between tree similarity and recombination rates measure at this scale (Fig. 4). While a relationship between recombination and phylogenetic discordance was predicted *a priori*, this negative result may stem from sampling recombination rates from only a single species (*Mus musculus*) and the fact that the rate of recombination is known to evolve quickly. Similar to findings in great apes (Hobolth et al. 2007), these results suggest that even high-resolution genetic resources from a single model species may be insufficient to help predict the landscape of discordance in a phylogenetic sample spanning 12 million years of evolution. Likewise, so called recombination hotspots identified in *M. musculus* also showed no difference relative to chromosome-wide levels of either initial tree similarity or rates of decay (Fig. 5B and C).

Evolutionary relationships around certain conserved genomic features may also be shaped by locally reduced effective population sizes due to a history of pervasive linked negative or positive selection. To test for this, we measured tree similarity in 10 kb windows around all genes, ultra-conserved elements (UCEs), and genes identified as evolving rapidly (*i.e.*, significantly elevated *dN*/*dS*) due to positive directional selection out to 1Mb and compared these patterns relative to chromosome-wide trends. We found that phylogenetic relationships around both genes and UCEs were more similar than chromosome-wide expectations (Fig. 5D). However, while regions around both genes and UCEs tended to show less discordance across short distances of a single 10kb window, tree similarity more rapidly decays to background levels for many genes. In contrast, decay rates around UCEs on average matched that of chromosome-wide averages, even out to 1 Mb (Fig. 5C), meaning that phylogenetic similarity is retained longer for UCEs than for genes. These results suggest that the history of strong purifying selection at UCE elements (Katzman et al. 2007) strongly skews patterns of discordance. Thus, phylogenetic inferences based on UCE markers would seem less prone to phylogenetic discordance and may provide cleaner estimates of species tree history, albeit while also potentially providing a highly misleading view of levels of genome-wide discordance. We also observed that regions close to rapidly evolving genes with a history of recurrent positive selection (see below) tended to exhibit less phylogenetic conservation relative to averages for all genes. However, we also observed higher variance around positively selected genes (Fig. 5D). Positive selection is often punctuated across lineages of a phylogeny while strong negative selection at UCEs is likely to be more pervasive, likely impacting the predictability of the influence of both forms of selection on discordance over evolutionary timescales.

This means that signals of directional positive selection on certain sites or lineages within a gene may be highly limited to those regions of the genome, with the reduced diversity associated with selective sweeps fading quickly over time, while the reduced constraint associated with gene regions in general being more widespread and permanent.

## Consequences of tree misspecification on analyses of protein evolution

Given that tree similarity decreases as a function of genomic distance, we next exeamined how this relates to the evolution of protein coding sequences. Among a set of 14,046 protein coding transcripts annotated from the *M. musculus* genome, we calculated that the average distance between the start and end of the coding sequence was 240.5 kb, or roughly 25 non-overlapping 10kb windows. At this distance, tree similarity is predicted to diminish considerably (e.g., by 0.093 wRF units), meaning that the phylogenetic history of individual genes may often contain phylogenetic discordance. We also found that out of 221,113 times the coding sequence in a gene overlapped with a 10 kb window, the inferred topology of the gene tree exactly matched the topology of the corresponding window tree only 10% of the time. This means that even when inferring gene trees on concatenated coding exons from a single transcript, a common practice, one is still likely averaging over multiple possible histories.

With knowledge of how tree similarity varies across the genome, we tested how tree misspecification might impact standard *dN*/*dS* based phylogenetic analyses for positive directional selection. Specifically, we used the common practice of assuming a single species tree for all genes and compared that to using individually inferred gene trees in three common statistical tests for positive selection: PAML’s M1a vs. M2a test, HyPhy’s BUSTED test, and HyPhy’s aBSREL test. We found evidence that tree misspecification likely induces both type I (false positives) and type II errors. Many genes were inferred as having experienced positive directional selection when using a single species tree, but not when using local gene trees and vice versa (Fig. 6A). Assuming the locally inferred gene tree is more accurate, this resulted in varying rates and types of error (Table 5). Specifically, for BUSTED, we observe that roughly 44% of genes inferred as having evolved under positive selection when using the gene tree are not inferred so when using the concatenated species tree (false negatives). The opposite is true for M1a vs. M2a, with 45% of genes showing signals of positive selection when using the concatenated species tree but not individual gene trees (false positives). For aBSREL, the proportion of false positives and false negatives was roughly equal around 12%. In general, genes found to be under positive selection using both tree types tended to be more concordant with the species tree than those that had evidence for positive selection either using only the concatenated tree or the gene tree (Fig. 6A).

# Discussion

Phylogenies provide insight into the relationships of species and serve as a framework for asking and answering questions about molecular and trait evolution. The extraordinary species richness of murine rodents complicates phylogenetic analyses because of the resources required to sample, sequence, and analyze such many widely distributed taxa. Earlier work either attempted to resolve specific groups such as *Mus* (Lundrigan et al. 2002; Suzuki et al. 2004) and *Apodemus* (Serizawa et al. 2000; Liu et al. 2004), or to uncover broader relationships across the subfamily (Martin et al. 2000; Steppan et al. 2005), in both cases using a limited number of nuclear or mitochondrial genes. Even so, evidence from these datasets suggested both phylogenetic discordance and mito-nuclear conflict across Murinae that lead to different genomic regions supporting incompatible phylogenetic reconstructions (Suzuki et al. 2004; Steppan et al. 2005; White et al. 2009).

Lecompte et al. (2008) provided one of the earliest well-supported phylogenetic reconstructions from across Murinae and the tribal classifications they proposed remain generally supported. More recent work has increased the number of taxa sampled, both for analyses of Murinae specifically (Pagès et al. 2016) and for their placement within Muroidea (Schenk et al. 2013; Steppan and Schenk 2017; Rowe et al. 2019), but the number of loci used for phylogenetic inference has remained low. Here, we leverage the resources of the model organisms the house mouse (*Mus musculus*) and the brown rat (*Rattus norvegicus*) along with new genomes from 8 closely related species and eight previously sequenced rodent genomes to understand the systematics of murine rodents and causes and consequences of phylogenetic discordance along the murine genome. These new genomes help to place these important model systems in an evolutionary context and begin to fill the gap in sampling of murine rodents which, despite their exceptional morphological and ecological diversity and species richness, have relatively few whole genomes sequenced. They further provide us with the resources to study the landscape of phylogenetic discordance across the genome.

We reconstructed a species tree of murine rodents based on thousands of UCEs from 18 species. The inferred tree (Fig. 1) mostly matches those inferred in previous studies, with notable exceptions being the placements of the *Apodemus* species and *R. soricoides.* We applied a range of strategies to assess the likelihood that this inference is the result of technical error, rather than true biological signal (see Supplemental Results), and find no evidence to suggest this is the case. Accounting for sequence errors (e.g., through the implementation of TreeMix (Mai and Mirarab 2018)), had no impact on inferred species tree topologies and did not substantially alter recovered quadripartition support nor gene tree discordance. We also note that, as Vanderpool et al. (2020) point out, when technical errors in alignment or model selection are allowed to propagate, discordance will be prevalent deeper in the tree. We instead observe discordance at shallow nodes and, as in the case of Chan et al (2020), discordance is greatest at short internal nodes (Fig. 1). This suggests there is value in investigating these patterns despite misgivings about aspects of the tree topology. Where phylogenetic reconstructions have made use of genomic markers - including UCEs - in other vertebrate taxa, gene tree discordance has been driven by a combination of incomplete lineage sorting or introgression (Alexander et al. 2017; Chan et al. 2020; Vanderpool et al. 2020; Alda et al. 2021), and methods to characterize these events are in active development (Hibbins and Hahn 2022). Using PhyloNetworks (Solis-Lemus et al. 2017) we see evidence of introgression into lineages exhibiting high conflict among their associated gene trees (between the Arvicanthini and Rhychomyini, and between the Murini and Praomyini. (Fig. S6), with the successive addition of hybrid edges improving the estimated likelihood of the resultant network.

When estimating divergence times on our inferred species trees, the Eumuroidea root (A) is placed at 24.27 Ma, which is concordant with the reconstruction of Schenk et al. (2013). The range between minimum and maximum reconstructed age is wide, overlapping both the maximum first appearance and estimated divergence time for the clade as described by Steppan and Schenk. (2017). The estimated date for Muridae (B) of 22.33 Ma likewise has a wide range, and appears to be approximately 2 (Schenk et al. 2013), 6 (Chevret and Dobigny 2005), or 7 (Steppan and Schenk 2017) million years older than other estimates. It aligns well with the estimated age of the clade recovered by Aghová et al. (2018) and, with some variance for the dating method used, the supermatrix derived mammalian tree of Meredith et al. (Meredith et al. 2011) but, as with other nodes, is much younger than was estimated by Hedges et al. (Hedges et al. 2015). The time of separation of Otomyini and Arvicanthi (P) (paraphyly of Arvicanthi notwithstanding, though see Fig. S3 for a time tree inferred without *Arvicanthis niloticus* and *Rhabdomys dilectus* that recovers a similar divergence time) is in general agreement with Lecompte et al. (2008), Schenk et al. (2013), and Aghová et al. (2018) at 8.17 Ma. Despite using a comparatively young calibration range for the *Rattus* group (Q) our results suggest the clade is possibly even younger given it is reconstructed at the minimum end of the assigned calibration (2.4 Ma), though this may better reflect an internal divergence within *Rattus* sensu lato than the origin of the group. We also reconstruct the origins of the Praomyini (K, 4.88 Ma) as approximately 0.5-1 Ma younger than other estimates (Lecompte et al. 2008; Schenk et al. 2013; Aghova et al. 2018), though these older estimates overlap our confidence intervals and Schenk et al. (2013) recover a close median age of 5.1 Ma. Nicolas et al. (2021) have recently estimated the Praomyini to have diversified even earlier (7.1 Ma). Within the Murini (M), our divergence of 3.55 Ma for *M. caroli* (N) followed by the separation of *M. musculus* and *M. spretus* (O, 1.58 Ma) is very similar to estimates by Suzuki et al. (2004).

Observing that discordance is prevalent among UCEs, we used the resources from the *M. musculus* model system to investigate genomic features that correlate with discordance. While our methods assume collinearity (i.e. no structural variation) and similar karyotypes of all genomes we acknowledge that both of these variations are known to occur in rodents (Stanyon et al. 1999; Yalcin et al. 2011; Romanenko et al. 2012; Keane et al. 2014) and could impact inferences along a linear chromosome in by bringing together distant regions of certain genomes in our analysis. However the overall impact of these types of structural variation should be minimized because, in testing over thousands of positions and loci within the genome, our methods average phylogenetic distance over varying genomic distances and loci. We find that discordance is widespread throughout the genome, even with a small sample of relatively closely related species. We find no correlation between levels of discordance along chromosomes and recombination rate, which is the driver of discordance caused by incomplete lineage sorting (ILS). We do find varying degrees of topological similarity around other features, such as genes, recombination hotspots, and UCEs. Regions of the genome around genes and UCEs are more similar than randomly chosen regions. While this similarity dissipates quickly around genes, returning to chromosome-wide levels of similarity, regions around UCEs remain more similar at long distances, up to 5Mb. This points to different scales of selective pressure around these conserved regions than the rest of the genome, with linked selection keeping these regions more similar than expected. With respect to rodent systematics, we find that despite the scale of discordance across chromosomes, the most frequent topology inferred among 10kb windows matches that recovered from concatenation of UCEs (Fig. 1). This implies that the inference of species relationships from conserved loci is still a valid method, and lends confidence to our inferred species tree despite the new placements of *Apodemus* and *R. soricoides*. Though caution is still warranted as only 13% of windows reflect this topology (Fig. 2A).

We also show that choice of tree topology drastically effects the results from various common tests for positive selection. It is known that tree misspecification can lead to mis-mapping of substitutions on the given tree (Hahn and Nakhleh 2016; Mendes and Hahn 2016), and we show that this mis-mapping results in both Type I and II errors. For each of the three selection tests run, HyPhy’s BUSTED and aBSREL and PAML’s M1a vs. M2a, while some genes showed evidence of positive selection whether the species tree or gene tree was used, many were unique to one tree or the other. The genes unique to the type of tree used were often discordant with the species tree while the genes that showed evidence of positive selection regardless of tree had levels of discordance comparable to all genes (85%, Figure 6, numbers in parentheses). This means that the mis-mapping of substitutions by supplying these tests with the wrong tree (i.e. the concatenated tree when gene trees are discordant) can lead to both false positives and false negatives when inferring genes under positive selection. Branch-site models, such as HyPhy’s BUSTED and aBSREL models which allow rates to vary among both branches and sites in the input data, result in more genes inferred with evidence for positive selection when using the correct tree (i.e., the gene tree, assuming no errors in gene tree reconstruction). This means that using a concatenated tree for these tests results in an increase in false negatives. On the other hand, models that only allow rates to vary among sites, such as PAML’s M1a vs. M2a test, show an increase in the number false positives inferred when using the wrong tree.

Our results have wide-ranging implications for various aspects of phylogenetics and comparative genomic analysis. First, it is imperative that when testing a specific locus for positive selection, discordance among loci must be accounted for. This most easily achieved by simply using the gene tree (or other locus type) as input to the test for selection (Mendes and Hahn 2016; Roycroft et al. 2021). However, as Mendes and Hahn (2016) point out, this may not completely mask the effects of discordance on substitution rates, as sites within a single gene may still have evolved under different histories because of within-gene recombination. We find evidence for this here as well, given that tree similarity between 10kb windows already starts to diminish and the average genomic distance between the beginning and end of a coding sequence in mice is 240.5 kb. Nevertheless, we recommend use of gene trees is whenever possible. After selection tests are run, it may still be useful to summarize them in the context of the species tree, which is complicated by the fact that by using gene trees that are discordant with the species tree, not every branch in the species tree will be represented in every gene. Methods to average rates per species branch can be implemented but must account for the degree of missing data from each branch by controlling for total number of sites tested.

Finally, because of recombination’s underlying contribution to phylogenomic discordance, one might be tempted to control for alternate topologies in a comparative genomics dataset by using a genetic map from a single, well studied species and sampling regions with low recombination rates. However, our results show that even among a small sample of 6 species, the use of a single recombination map is insufficient to control for discordance. This is likely because the recombination rate evolves as well and is linked to structural variation (Morgan et al. 2017) which is unaccounted for with use of a single reference genome, resulting in a diminished signal of correlation between rates and phylogenetic discordance over time. This would be compounded for more diverged sample sets. Genetic maps from multiple species may aid in localizing recombination’s effects on phylogenetic discordance, if common segments of low recombination could be identified, but this may still be difficult for most studies’ sampling. Even in murine rodents, which have 2 species with high quality genetic maps, we were unable to perform such a cross-species recombination analysis because of the lack of collinearity between the *Mus musculus* and the *Rattus novegicus* genomes.

Our results increase whole genome sampling in the diverse group of murine rodents and provide a broad murine species tree based on thousands of UCE loci. The high-quality *M. musculus* genome allows us to show for the first time the fine-scale patterns and effects of phylogenetic discordance along chromosomes, which highlights an underappreciated complexity of comparative genomics. We demonstrate how common tests for selection can be prone to error if only a single species tree is used and recommend ways to overcome this by using gene trees for any locus-based analysis. Using our results, we can better understand the complexities of phylogenomic datasets which will help to ensure that steps are taken to accommodate these details in comparative studies going forward.

# Tables

### Table 1: All taxa whose genomes were included in this study, the source of the assembly, and the assembly level of each genome.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Taxon** | **Assembly source** | **Assembly level** | **No. UCEs** | **Used in genome-wide discordance analyses** |
| *Apodemus speciosus* | GenBank: GCA\_002335545.1 | Scaffolds | 2223 |  |
| *Apodemus sylvaticus* | GenBank: GCA\_001305905.1 | Scaffolds | 2381 |  |
| *Arvicanthis niloticus* | GenBank: GCA\_011762505.1 | Chromosomes | 2422 |  |
| *Grammomys dolichurus* | This study: Aligned to mm10 reference | Chromosomes | 2644 | \* |
| *Hylomyscus alleni* | This study: Aligned to mm10 reference | Chromosomes | 2645 | \* |
| *Mastomys natalensis* | This study: Aligned to mm10 reference | Chromosomes | 2643 | \* |
| *Mus caroli* | GenBank: GCA\_900094665.2 | Chromosomes | 2512 |  |
| *Mus musculus* | GenBank: GRCm38.p6/mm10 | Chromosomes | 2645 | \* |
| *Mus pahari* | GenBank: GCA\_900095145.2 | Chromosomes | 2456 |  |
| *Mus spretus* | GenBank: GCA\_001624865.1 | Chromosomes | 2533 |  |
| *Otomys typus* | This study: De novo assembled | Scaffolds | 2491 |  |
| *Phodopus sungorus* | Moore et al., 2022: De novo assembled | Chromosomes | 2441 |  |
| *Praomys delectorum* | This study: Aligned to mm10 reference | Chromosomes | 2645 | \* |
| *Rattus norvegicus* | GenBank: GCA\_015227675.2 | Chromosomes | 2292 |  |
| *Rattus rattus* | GenBank: GCA\_011064425.1 | Chromosomes | 2312 |  |
| *Rhabdomys dilectus* | This study: Aligned to mm10 reference | Chromosomes | 2645 | \* |
| *Rhombomys opimus* | GenBank: GCA\_010120015.1 | Scaffolds | 2396 |  |
| *Rhynchomys soricoides* | This study: Aligned to mm10 reference | Chromosomes | 2643 | \* |

### Table 2: Prior node ages used in phylogenetic dating in millions of years before present.

|  |  |  |  |
| --- | --- | --- | --- |
| **Clade** | **Minimum Age** | **Maximum Age** | **Citation** |
| *Mus* | 5.3 | 7.2 | Steppan and Schenk, 2017 |
| *Apodemus* | 5.3 | 7.2 | Schenk et al., 2013 |
| *Rattus* | 2.4 | 3.6 | Steppan and Schenk, 2017 |
| Murinae | 12.1 | 14.05 | Schenk et al., 2013 |

### Table 3: The most frequently recovered topologies across all 10kb windows. RS = *Rhyncomys soricoides*, GD = *Grammomys dolichurus*, RD = *Rhabdomys dilectus*, MM = *Mus musculus*, HA = *Hylomyscus alleni*, MN = *Mastomys natalensis*, PD = *Praomys delectorum*.

|  |  |  |  |
| --- | --- | --- | --- |
| Rank | Topology | # of windows | Proportion of windows |
| 1\* | (RS,((GD,RD),(MM,(HA,(MN,PD))))); | 20581 | 0.125 |
| 2 | (RS,((GD,RD),(MM,((HA,MN),PD)))); | 16616 | 0.101 |
| 3 | (RS,((HA,(MN,PD)),(MM,(GD,RD)))); | 14505 | 0.0883 |
| 4 | (RS,(MM,((GD,RD),(HA,(MN,PD))))); | 10964 | 0.0668 |
| 5 | (RS,(((HA,MN),PD),(MM,(GD,RD)))); | 9717 | 0.0592 |
| 6 | (RS,(GD,((HA,(MN,PD)),(MM,RD)))); | 8909 | 0.0543 |
| 7 | (RS,(MM,((GD,RD),((HA,MN),PD)))); | 7438 | 0.0453 |
| 8 | (RS,((GD,RD),(MM,((HA,PD),MN)))); | 7129 | 0.0434 |

\*The topology recovered from concatenation of genes or UCEs

### Table 4: Summaries of phylogenies per chromosome.

|  |  |  |
| --- | --- | --- |
| Chromosome | Top 3 recovered topologies\* | # of unique topologies recovered |
| 1 | 1,2,3 | 315 |
| 2 | 1,2,3 | 263 |
| 3 | 1,3,8 | 269 |
| 4 | 1,2,5 | 298 |
| 5 | 1,3,2 | 325 |
| 6 | 1,2,3 | 323 |
| 7 | 1,2,3 | 388 |
| 8 | 1,2,3 | 267 |
| 9 | 1,2,3 | 233 |
| 10 | 1,3,2 | 250 |
| 11 | 1,2,3 | 199 |
| 12 | 1,2,3 | 330 |
| 13 | 1,2,3 | 313 |
| 14 | 1,2,3 | 362 |
| 15 | 1,3,2 | 217 |
| 16 | 1,2,3 | 222 |
| 17 | 1,2,3 | 328 |
| 18 | 1,2,3 | 171 |
| 19 | 1,2,3 | 160 |
| X | 7,5,4 | 346 |

\*Ranks correspond to those defined in Table 1.

### Table 5: Rates and types of error when using concatenated trees for gene-based selection tests.

|  |  |  |
| --- | --- | --- |
| Test | False positive rate | False negative rate |
| BUSTED | 11.3% | 43.5% |
| aBSREL | 11.4% | 13.8% |
| M1a vs. M2a | 45.0% | 1.8% |

# Figure Legends

### Figure 1

Species trees inferred from concatenation of ultra-conserved elements (UCEs) from 18 rodent species. Internal nodes are labeled by a letter identifier referenced in the text and site and gene concordance factors (e.g. Label: sCF/gCF) as well as a bar indicating the confidence interval for divergence time estimation. Bootstrap/SH-aLRT values were all 100. Bottom scale represents time in millions of years before present. Fossil calibrations are described in Tables 2 and S2, with Node C used as a fixed calibration point. The sub-family Murinae is highlighted on the right, with Tribes being labeled following the classifications used by Lecompte et al. (2008). New genomes presented in this study are underlined, and genomes used for the genome-wide phylogenetic discordance analysis are indicated with an asterisk.

### Figure 2

The landscape/profile of phylogenetic discordance across non-overlapping 10kb windows in murine genomes. A) Distribution of the 20 most frequent topologies recovered across all windows. Numbers above bars indicate proportion of each topology. B) The top three topologies recovered on chromosome 1. C) Distribution of the top three topologies recovered along chromosome 1. The x-axis is scaled to the length of the chromosome and each vertical bar represents one 10kb window. The three most frequent topologies occupy the first three rows while all other topologies are shown in the bottom row.

### Figure 3

Similarity between 10kb windows decays as genomic distance between windows increases. A) The log fit to the mean of distributions of tree distances between windows at increasing genomic distance (10kb steps). Each line represents one chromosome. B) The same, but on a log scale with a linear fit. C) For every window on each chromosome, the genomic distance between windows at which tree distance becomes random was measured and averaged. This process was repeated 100 times. 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 genome.

### Figure 4

No correlation between tree similarity and recombination rate in 5Mb windows. A) Tree similarity as measured by the wRF distance between the first and last 10kb windows within the 5Mb window. B) The rate at which tree similarity from 10kb windows decreases over a 5Mb window.

### Figure 5

Differences in tree similarity up to 5Mb around genomic features. A) Log fits between the distance between 10kb windows centered on genes, UCEs, or recombination hotspots and other 10kb windows and increasing genomic distance and tree similarity for chromosome 7. Points represent the mean of all features at that distance. B) The same, but on a log scale with a linear fit. C) The rate of decreasing tree similarity with genomic distance for each of the three features and the chromosome-wide averages. Each point represents the slope for a single chromosome. D) The tree distance between the 10kb window centered on the feature and the adjacent 10kb window for each of the three features and the chromosome-wide averages. Each point represents the average of all adjacent distances for a single chromosome. E) For each genomic distance, the difference between the average tree similarity around each of the four features and the chromosome-wide average.

### Figure 6

Tree misspecification leads to erroneous results in tests for positive selection. A) The proportion of genes inferred to be under positive selection for three tests using either a single species tree (concatenated tree) or individual gene trees, as well as those found in both cases (shared). Numbers in the bars indicate raw counts, and percentages indicate the percent of genes in that category that are discordant from the species tree. B) The overlap between genes found under positive selection between the three tests using individual gene trees. C) The overlap between genes found under positive selection between the three tests using the species tree.

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